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Evaluation of Stable Labeled Compounds as
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EVALUATION OF STABLE LABELED COMPOUNDS AS
INTERNAL STANDARDS FOR
QUANTITATIVE GC/MS DETERMINATIONS

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16. ABSTRACT The report gives results of an investigation of the use of stable isotopically labeled compounds as internal standards for quantitative isotope dilution GC/MS determinations. The availability of labeled compounds and the costs associated with using them for routine analyses were evaluated using the volatile, acid, and base/neutral fraction priority pollutants as a target component base. About 75 percent of these compounds are available as appropriate stable isotopically labeled analogs. Incorporating the stable labeled compounds as part of an analysis protocol is expected to decrease overall analysis costs by 30-70 percent when recommended quality assurance procedures are employed. Fundamental approaches to measurement parameter selection were identified which indicate the best ion fragment masses to measure, the best labeled compound spiking concentration, and the most appropriate data reduction methods.		
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ABSTRACT

This research program was initiated with the overall objective of evaluating the use of stable isotopically labeled organic compounds to act as quantitative internal standards for gas chromatographic/mass spectrometric (GC/MS) determinations.

The use of stable isotopically labeled compounds as internal standards for quantitative isotope dilution GC/MS determinations was investigated. The availability of labeled compounds and the costs associated with using them for routine analyses was evaluated using the volatile, acid, and base/neutral fraction priority pollutants as a target component base. At present, approximately 75 percent of these compounds are available as appropriate stable isotopically labeled analogs. Incorporation of the stable labeled compounds as part of an analysis protocol is expected to decrease overall analysis costs by 30 to 70 percent when recommended quality assurance procedures are employed. Fundamental approaches to measurement parameter selection were identified which indicate (1) the best ion fragment masses to measure, (2) the best labeled compound spiking concentration, and (3) the most appropriate data reduction methods.

Subsequent to the fundamental investigations, twenty industrial effluents were analyzed to establish the analytical precision and accuracy of isotope dilution. The samples were also analyzed using a conventional internal standard approach. This study involved 36 priority pollutants spiked into the test samples. On the average, isotope dilution yielded calculated percent recoveries of from 100.0 percent for priority pollutants in the base/neutral fraction to 98.5 percent for those in the volatile fraction. The acid fraction data resulted in 99.1 percent calculated recovery. These values compare 56.3, 77.4 and 51.6 percent for recovery the same priority pollutants as determined in their respective fractions using the conventional internal standard quantitation approach. Analytical precision was, likewise, better using isotope dilution where 12.1, 19.0 and 9.2 percent standard deviations were obtained for the base/neutral, acid and volatile fractions, respectively, compared with 57.3, 67.5 and 39.5 percent standard deviation for the conventional internal standard approach data.

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SECTION 1

INTRODUCTION

The requirement for qualitative and quantitative determinations of trace organic materials in effluent streams has been established by both the EPA Environmental Assessment Programs and the EPA's response to the 1976 Consent Decree. The use of gas chromatographic/mass spectrometric (GC/MS) analysis for this purpose has received general acceptance.

One of the most desirable features of GC/MS analysis is the ability to obtain highly specific qualitative and quantitative analytical information in a single analysis. The qualitative aspects of the technique are extremely well proven. Quantitatively, however, GC/MS results have often been rather disappointing in terms of precision, accuracy and cost-effectiveness. The cost-effectiveness of quantitative GC/MS determinations is severely impacted when the sample must also be analyzed separately to evaluate compound recovery from the original sample matrix. This has been a particular problem of concern with the determination of priority pollutants in industrial waste waters¹ where recovery from waste waters has been highly variable. As a consequence, quality assurance procedures involving multiple sample preparations and analysis have been instituted as a means to evaluate precision and accuracy. These procedures have increased the cost per analysis by approximately a factor of two, and do not provide any improvement in the quality of the data, only a check on that quality.

Similar problems with the qualitative and quantitative GC/MS analyses have been encountered in other fields where sample media are extremely complex. Perhaps the most notable of these is the field of pharmacology and the study of drug-related phenomena in tissue and body fluids. The complexity and variation of biological samples, like environmental samples, have resulted in a large number of GC/MS investigations. Because poor analytical results can have an immediate impact on human health in some of these pharmacological studies, reliable high-quality data have been essential. One of the routes to these data has been the use of stable isotopically labeled analogs of the compounds under investigation (target compounds) to act as internal standards for quantitation². With this approach, called isotope dilution, the labeled analog of the target compound is added to the sample prior to preparation. Because the labeled and unlabeled materials are actually the same compound, the recovery of one can be directly inferred by the recovery of the other. Consequently,

separate, distinct analyses of spiked samples are not necessary and hence, costs are reduced. It has been demonstrated that this approach to GC/MS quantitation for biological samples provides precisions and accuracies on the order of a few percent over concentration ranges of up to three orders of magnitude³. A logical extension of this technology was to extend these applications to environmental samples where it was expected to provide equivalent good results.

SECTION 2

CONCLUSIONS

The use of stable isotopically labeled internal standards for quantitative isotope dilution GC/MS determinations has been shown to be more accurate and more precise than conventional internal standard quantitation approaches in applications to the measurement of priority pollutants in industrial waste waters. The isotope dilution method was found to be free of matrix effects which reduce the accuracy of other GC/MS analysis methods and to be equally precise and accurate for measurements made in two different laboratories. Finally, incorporation of labeled analogs of compounds of interest into analysis mixtures can be expected to reduce overall analysis cost because quality assurance functions can be incorporated into each sample analysis rather than into the analysis of additional samples.

SECTION 3

RECOMMENDATIONS

Although the study reported here clearly demonstrated quantitative isotope dilution GC/MS of great potential value, several aspects of the method should still be investigated. These are:

- A full interlaboratory testing of the methods needs to be carried out using "blind" samples
- The long-term stability of labeled compounds, especially when contained in spiking solution media, should be investigated.
- The priority pollutant methods should be expanded to include data for all base/neutral, acid and volatile fraction compounds.
- Isotope dilution should be investigated for potential application to pesticide determinations.
- Some available labeled analogs, such as methylene chloride-d₂, 1,1-dichloroethylene-d₂, and 2,4,6-trichlorophenol-d₂, are not ideal labeled analogs; alternatives should be identified.

SECTION 4

COST AND AVAILABILITY OF STABLE LABELED COMPOUNDS

Because a complete compilation of all compounds of environmental significance does not exist, the organic priority pollutants covered by the Consent Decree were selected as a representative group of compounds in order to perform a meaningful appraisal of cost and availability. These compounds were selected for the following reasons:

- Priority pollutants cover a broad range of compound types and consequently, provide a representative base for comparison.
- Matrix effects associated with recovery of priority pollutants during sample preparation are known to exist and to result in limitations to accurate quantitation.
- Priority pollutants are currently under investigation using quantitative GC/MS measurement techniques.

Availability and material costs were assessed on a per-compound basis through discussions with major commercial distributors of stable labeled compounds, these included:

- Merck, Sharp and Dohme
- Prochem Isotopes
- KOR Isotopes
- Stohler Isotope Chemicals
- Isotope Labeling Corporation
- Monsanto Research Company Isotope Sales

Table 1 presents the material cost estimates per analysis for the 29 deuterium labeled priority pollutants which were available from major commercial distributors of stable labeled compounds as of September 1979. These material cost estimates were obtained from the most recent catalogs supplied by the isotope distributors and through discussions with representatives of these companies. The estimates were based on an

TABLE 1

MATERIAL COST PER ANALYSIS FOR CURRENTLY AVAILABLE
DEUTERIUM LABELED PRIORITY POLLUTANTS

BASE/NEUTRAL AND ACID			
Compound	Cost For (100 μ g (\$))	Isotopic Purity	Isotope Distributor
<u>Base/Neutral</u>			
Acenaphthene, d_{10}	4.00×10^{-3}	98	ILC
1,2-Dichlorobenzene, d_4	2.50×10^{-3}	98	MK,ST
1,4-Dichlorobenzene, d_4	5.80×10^{-3}	98	MK
1,3,5-Trichlorobenzene, d_3	15.5×10^{-3}	99	MK
Naphthalene, d_{10}	1.70×10^{-3}	98	ALD, ALF
Nitrobenzene, d_5	0.32×10^{-3}	99	ALD, ALF
Benzo(a)anthracene, d_{12}	30.6×10^{-3}	99	MK
Benzo(a)pyrene, d_{12}	135×10^{-3}	98	MK
Chrysene, d_{12}	15.1×10^{-3}	98	MK,ST
Anthracene, d_{10}	4.24×10^{-3}	98	MK,ST
Phenanthrene, d_{10}	5.30×10^{-3}	98	MK,ST
Fluorene, d_{10}	30.0×10^{-3}	98	ST
Pyrene, d_{10}	22.5×10^{-3}	98	MK
<u>Acid</u>			
Phenol, d_6	3.70×10^{-3}	98	ALD,ALF

TABLE 1 (Continued)

VOLATILES			
Compound	Cost For 150 ng (\$)	Isotopic Purity	Isotope Distributor
Acrylonitrile, d_3	10.8×10^{-6}	98	ST
Benzene, d_6	0.30×10^{-6}	99.5	ALD,MK,ST
Chlorobenzene, d_5	4.5×10^{-6}	99	ALD,ALF
1,2-Dichloroethane, d_4	1.55×10^{-6}	99	MK,ST
1,1,2-Trichloroethane, d_1	30×10^{-6}	98	MK
1,1,1-Trichloroethane, d_3	30×10^{-6}	98	MK
1,1,2,2-Tetrachloroethane, d_2	0.45×10^{-6}	98	ST
Chloroethane, d_5	13.7×10^{-6}	99	MK
Chloroform, d_1	0.025×10^{-6}	99.8	ALD,ALF
Ethylbenzene, d_{10}	1.5×10^{-6}	98	ALD,ALF
Methylene Chloride, d_2	1.00×10^{-6}	99	ALD
Methyl Chloride, d_3	12.2×10^{-6}	99	MK
Methyl Bromide, d_3	1.95×10^{-6}	99.5	ST
Bromoform, d_1	0.33×10^{-6}	99	MK,ST
Toluene, d_8	0.60×10^{-6}	99	MK,ST

Key: ST = Stohler Isotopes
 PR = Prochem Isotopes
 MK = Merck and Co.
 ILC = Isotope Labeling Corp.
 ALF = Alfa Chemicals
 ALD = Aldrich Chemicals

assumption that 100 μ g of each extractable compound would be added to the sample for each 1-liter sample extracted and 150 ng of each purgeable compound would be added to the sample for each 5-ml sample purged.

Although deuterated compounds are generally less costly than other stable labeled compounds, their application is limited in some instances due to isotopic exchange (see Section 5). For this reason, the cost and availability of non-deuterium labeled priority pollutants was also investigated. Table 2 presents the material cost estimates per analysis for 15 non-deuterium labeled priority pollutants which were available from major isotope distributors as of September 1979.

In addition to material cost estimates for stable labeled compounds which were available, cost estimates were obtained for the synthesis of compounds which were not currently available. The custom synthesis cost for deuterium labeled priority pollutants was estimated to be approximately \$1,000/gram from Stohler Isotope Chemicals. The price of 1 gram of carbon-13 labeled material from California Bionuclear Corporation ranged from about \$1,000 for the less complex compounds such as benzene and toluene to about \$3,000 for the more complex compounds. The costs varied for deuterium labeled and carbon-13 labeled compounds, depending on the position of the label, the number of labeled sites, the type of synthesis required, and the isotopic purity of the final compound. The materials cost estimates per analysis for deuterium and non-deuterium labeled internal standards are summarized in Table 3 and 4, respectively.

During the course of this study, the availability of labeled priority pollutants increased. At the start, 9, 28 and 48 percent of the acid, base/neutral and volatile fraction compounds, respectively, were available. At present, 100, 62 and 87 percent of those fractions priority pollutants are available, respectively.

TABLE 2
MATERIAL COST PER ANALYSIS FOR CURRENTLY AVAILABLE
NON-DEUTERIUM LABELED PRIORITY POLLUTANTS

BASE/NEUTRALS AND ACID			
Compound	Cost For 100 μ g (\$)	Isotopic Purity	Isotope Distributor
<u>Base/Neutral</u>			
Benzidine, $^{15}\text{N}_1$	70.0 10^{-3}	99	ST
Nitrobenzene, $^{15}\text{N}_1$	7.50 10^{-3}	99	PR
Fluorene, $^{13}\text{C}_9$	275 10^{-3}	90	KOR,ST
<u>Acid</u>			
Phenol, $^{13}\text{C}_6$	180 10^{-3}	90	KOR
VOLATILES			
Compound	Cost For 100 ng (\$)	Isotopic Purity	Isotope Distributor
Benzene, $^{13}\text{C}_6$	143 10^{-3}	90	MK,PR,ST
1,2-Dichloroethane, $^{13}\text{C}_2$	165 10^{-3}	90	ST
Chloroform, $^{13}\text{C}_1$	9.45 10^{-3}	90	PR
Carbon Tetrachloride, $^{13}\text{C}_1$	2.93 10^{-3}	90	KOR
Ethyl benzene, $^{13}\text{C}_1$	186 10^{-3}	90	MK
Methylene Chloride, $^{13}\text{C}_1$	147 10^{-3}	90	KOR
Methyl Chloride, $^{13}\text{C}_1$	117 10^{-3}	90	MK
Methyl Bromide, $^{13}\text{C}_1$	2.93 10^{-3}	90	MK,PR
Bromoform, $^{13}\text{C}_1$	147 10^{-3}	90	KOR
Toluene, $^{13}\text{C}_1$	5.25 10^{-3}	90	KOR
Chlorobenzene, $^{35}\text{C}_1$	105 10^{-3}	90	ST

Key: ST = Stohler Isotope MK = Merck & Co.
PR = Prochem/Isotopes KOR = KOR Isotopes

TABLE 3

SUMMARY OF MATERIAL COSTS PER ANALYSIS FOR
DEUTERIUM LABELED PRIORITY POLLUTANTS*

Fraction	Estimated Cost (\$)	Number of Compounds
Currently Available Compounds		
Base/Neutral	0.273	13
Acid	0.0740	1
Volatile	0.000110	15
Total	0.347	29
Custom Synthesis Compounds		
Base/Neutral	3.10	31
Acid	0.900	9
Volatile	0.00180	12
Total	4.00	52
Currently Available and Custom Synthesis Compounds		
Base/Neutral	3.37	44
Acid	0.974	10
Volatile	0.00191	27
Total	4.34	81

*As of September 1979

TABLE 4
SUMMARY OF MATERIAL COSTS PER ANALYSIS FOR
NON-DEUTERIUM LABELED PRIORITY POLLUTANTS*

Fraction	Estimated Cost (\$)	Number of Compounds
Currently Available Compounds		
Base/Neutral	0.353	3
Acid	0.180	1
Volatile	0.00122	11
Total	0.534	15
Custom Synthesis Compounds		
Base/Neutral	11.6	44
Acid	2.0	10
Volatile	0.00360	20
Total	13.6	74
Currently Available and Custom Synthesis Compounds		
Base/Neutral	12.0	47
Acid	2.18	11
Volatile	0.00482	31
Total	14.2	89

*As of September 1979

SECTION 5

DEUTERIUM LABEL EXCHANGE

When deuterated compounds are used as internal standards, the potential for label exchange (deuterium exchange) exists and could lead to errant results. The extent of this potential problem is expected to vary primarily as a function of sample pH. Highly basic or acidic samples would promote deuterium exchange in the same way that they would promote proton exchange; neutral solutions would tend to reduce exchange but not necessarily eliminate it. The potential for exchange with ^{13}C , ^{15}N , and ^{18}O labeled compounds is essentially nonexistent because major molecular bonds would be altered in order for exchange to take place.

Because exchange is so unlikely a problem with labels other than deuterium (D), exchange studies were confined to D labeled compounds. The most cost-effective way to study D exchange was to evaluate the rates and sites at which D's from solution replace H's on unlabeled molecules. This was accomplished by preparing synthetic mixtures containing unlabeled compounds in the deuterated analog of water, D_2O , and preparing these mixtures in the conventional way except for substituting NaOD and DCl when appropriate. The resulting sample fractions were analyzed by GC/MS and the spectra evaluated by comparison with the unlabeled standards which have not been exposed to deuterium oxide. Differences in the mass spectra were used to indicate exchange. When exchange was noted, the spectra were used to identify and specify unacceptable label location. The main thrust of this investigation was to identify the potential label locations appropriate for use in internal standard applications for priority pollutant determinations.

Experimental studies for isotope exchange were carried out for the three nonpesticide priority pollutant fractions by spiking solutions of D_2O with naturally abundant priority pollutants and evaluating the mass spectra of subsequent sample extracts for evidence of proton-to-deuterium exchange. Spiked D_2O samples were stored at 4° and 25°C for 0, 48 and 96 hours prior to extraction. Also, the pD (equivalent to pH for H_2O) was adjusted to give storage conditions at pD's of 2, 7 and 12. In this way, more severe conditions than those encountered in performing a separatory funnel shake-out or continuous liquid-liquid extraction were tested. The results of these experiments are given in Table 5. It should be noted that not all compounds were available in sufficient quantities for testing at the time these studies were carried out. However, most compounds were tested and of these, only six gave evidence of exchange. The specific nature of this exchange is shown in Table 6.

TABLE 5
RESULTS OF ISOTOPE EXCHANGE STUDIES

BASE/NEUTRAL		
No Detectable Exchange	Detectable Exchange	Not Detected
2,4,6-trichlorophenol benzidine 1,2,4-trichlorobenzene hexachlorobenzene hexachloroethane 2-chloronaphthalene 1,2-dichlorobenzene 1,3-dichlorobenzene 1,4-dichlorobenzene 1,4-dichlorobenzidine 2,4-dinitrotoluene 2,6-dinitrotoluene 1,2-diphenylhydrazine (as azobenzene) fluoranthene 4-chlorophenylphenylether 4-bromophenylphenylether hexachlorobutadiene isophorone naphthalene nitrobenzene N-nitrosodiphenylamine bis(2-ethylhexyl)phthalate butylbenzylphthalate di-n-butylphthalate diethylphthalate dimethylphthalate benzo(a)anthracene benzo(a)pyrene benzo(b)fluoranthene benzo(k)fluoranthene chrysene acenaphthylene anthracene phenanthrene pyrene		bis(2-chloroethyl) ether bis(2-chloroisopropyl)ether ² bis(2-chloroethoxy) methane ² hexachlorocyclopentadiene N-nitrosodimethylamine N-nitrosodi-n-propylamine di-n-octylphthalate ² benzo(ghi)perylene ² dibenzo(a,h)anthracene ² indeno(1,2,3-cd)pyrene ² 2,3,7,8-tetrachlorodibenzo-p-dioxin ²

Note: ¹ Only exchange of the non-phenolic protons was considered for acid compounds.

² Compound not present in test mixture.

TABLE 5 (Continued)

ACID COMPOUNDS ¹		
No Detectable Exchange	Detectable Exchange	Not Detected
2,4,6-trichlorophenol p-chloro-m-cresol 2-chlorophenol 2,4-dichlorophenol 2,4-dimethylphenol 2-nitrophenol 4-nitrophenol 2,4-dinitrophenol 4,6-dinitro-o-cresol pentachlorophenol phenol		
VOLATILE COMPOUNDS		
methylene chloride 1,1-dichloroethylene 1,1-dichloroethane carbontetrachloride 1,2-dichloropropane 1,1,2-trichloroethane tetrachloroethylene chlorobenzene trichlorofluoromethane trans-1,2-dichloroethylene 1,2-dichloroethane 1,1,1-trichloroethane trans-1,3-dichloropropene cis-1,3-dichloropropene benzene 1,1,2,2-tetrachloroethane toluene ethylbenzene	chloroform dichlorobromomethane chlorodibromomethane bromoform trichloroethylene	chloromethane ² dichlorodifluoro- methane ² bromomethane ² vinyl chloride ² chloroethane ² acrolein ² acrylonitrile ² bis(chloromethyl) ether 2-chloroethylvinyl- ether

Note: ¹ Only exchange of the non-phenolic protons was considered for acid compounds.

² Compound not present in test mixture.

TABLE 6
PERCENT DEUTERIUM EXCHANGE

Storage Conditions Compound	Time (hr)		96				48			0	
	Temperature (C°)		25				4			25	
	pD		2	7	12		2	7	12	7	7*
Chloroform			16	90	100		18	26	100	25	26
Dichlorobromomethane			2	100	100		3	50	99	13	20
Chlorodibromomethane			6	95	99		7	90	99	43	62
Bromoform			25	84	95		34	100	100	95	99
Trichloroethylene			2	11	97		2	3	92	2	3
Fluorene			16	18	26		10	41	72	13	16

*pH; this sample contained no D₂O and should be considered as a reference for which no exchange took place. Percent exchange values exist due to the presence of low abundance nonisotope-related ion fragment.

SECTION 6

ISOTOPE LABEL DETAIL

Using isotope exchange data, electron ionization fragmentation pattern interpretations, etc., it was possible to identify appropriate labels and label locations for the priority pollutants. For those molecules without specific exchange data, recommendations are based on inference from homologous compounds. For example, all polynuclear aromatic compounds (PNA's) were not available in sufficient quantity to test but, because no exchange of aromatic protons was observed in the PNA's tested, these locations were presumed not to undergo exchange in the untested PNA's. Also, when the precise fragmentation of a molecule was unclear, such as was the case with isophorone, predeuteration or ^{13}C labeling was recommended. The results of this effort are tabulated in Table 7.

TABLE 7
RECOMMENDED ISOTOPE LABEL DETAIL

ACID COMPOUNDS			
Name	Label	Number of Labels	Location
2,4,6-trichlorophenol	D	2	ring
p-chloro-m-cresol	D	2-3	ring
2-chlorophenol	D	2-4	ring
2,4-dichlorophenol	D	2-3	ring
2,4-dimethylphenol	D	2-3	ring
2-nitrophenol	D	2-4	ring
4-nitrophenol	D	2-4	ring
2,4-dinitrophenol	D	2-3	ring
4,6-dinitro-o-cresol	D	2	ring
pentachlorophenol	¹³ C	2-6	ring
phenol	D	2-5	ring

TABLE 7 (Continued)

BASE/NEUTRAL COMPOUNDS			
Name	Label	Number of Labels	Location
acenaphthene	D	2-10	any
benzidine	D	2-12	any
1,2,3-trichlorobenzene	D	2-3	any
hexachlorobenzene	¹³ C	2-6	any
hexachloroethane	¹³ C	2	any
bis(2-chloroethyl)ether*	D	4	1 position on ethyl's
2-chloronaphthalene	D	2-7	any
1,2-dichlorobenzene	D	2-4	any
1,3-dichlorobenzene	D	2-4	any
1,4-dichlorobenzene	D	2-4	any
3,3'-dichlorobenzidine	D	2-10	any
2,4-dinitrotoluene	D	2-3	ring
2,6-dinitrotoluene	D	2-3	ring
1,2-diphenylhydrazine	D	4-10	Equal number on each ring
fluoranthene	D	2-10	any
4-chlorophenylphenylether	D	2-9	any
4-bromophenylphenylether	D	2-9	any
bis(2-chloroisopropyl)ether**	--	---	---
bis(2-chloroethoxy)methane*	D	10	all
hexachlorobutadiene	¹³ C	2-4	any
hexachlorocyclopentadiene	¹³ C	5	all
isophorone	D	14	all
naphthalene	D	2-8	any
nitrobenzene	D	2-4	any
N-nitrosodimethylamine*	D	6	all
N-nitrosodiphenylamine	D	4-10	Equal number on each ring
N-nitrosodi-n-propylamine**	--	---	---

*assuming no exchange
TABLE 7 (continued)

**insufficient data

TABLE 7 (Continued)

BASE/NEUTRAL COMPOUNDS (Continued)			
Name	Label	Number of Labels	Location
bis(2-ethylhexyl)phthalate	D	2-4	ring
butylbenzylphthalate	D	2-4	ring
di-n-butylphthalate	D	2-4	ring
di-n-octylphthalate	D	2-4	ring
diethylphthalate	D	2-4	ring
dimethylphthalate	D	2-4	ring
benzo(a)anthracene	D	2-12	any
benzo(a)pyrene	D	2-12	any
benzo(b)fluoranthene	D	2-12	any
benzo(k)fluoranthene	D	2-12	any
chrysene	D	2-12	any
acenaphthylene	D	2-8	any
anthracene	D	2-10	any
benzo(ghi)perylene	D	2-12	any
fluorene	D	2-6	Aromatic positions only
phenanthrene	D	2-10	any
dibenzo(a,h)anthracene	D	2-14	any
indeno(1,2,3-cd)pyrene	D	2-12	any
pyrene	D	2-10	any
2,3,7,8-tetrachlorodibenzo-p-dioxin	¹³ C	3-12	any

*assuming no exchange

**insufficient data

TABLE 7 (continued)

TABLE 7 (Continued)

VOLATILE COMPOUNDS			
Name	Label	Number of Labels	Location
acrolein*	D	2-4	any
acrylonitrile*	D	2-3	any
benzene	D	2-6	any
carbon tetrachloride	¹³ C	1	any
chlorobenzene	D	2-5	only
1,2-dichloroethane	D	4	all
1,1,1-trichloroethane	D	2-3	any
1,1-dichloroethane	D	4	all
1,1,2-tetrachloroethane	D	3	all
1,1,2,2-tetrachloroethane	D	2	all
chloroethane	D	5	all
bis(chloromethyl)ether*	D	2-4	any
2-chloroethylvinylether*	D	2-7	any
chloroform	¹³ C	1	only
1,1-dichloroethylene	D	2	only
1,2-trans-dichloroethylene	D	2	only
1,2-dichloropropane	D	2-6	any
1,3-dichloropropylene	D	2-4	any
ethylbenzene	D	2-10	any
methylene chloride	D	2	only
methyl chloride*	D	2-3	any
methyl bromide*	D	2-3	any
bromoform	¹³ C	1	only
dichlorobromomethane	¹³ C	1	only
trichlorofluoromethane	¹³ C	1	only
dichlorodifluoromethane	¹³ C	1	only
tetrachloroethylene	¹³ C	2	only
toluene	D	2-8	any
trichloroethylene	¹³ C	2	only
vinyl chloride*	D	2-3	any

*assuming no exchange

**insufficient data

SECTION 7

COST COMPARISON FOR QUALITY ASSURANCE

An estimate of costs associated with the protocol used for priority pollutant verification was made for a "typical" verification episode (Table 8) as described by Dr. Dale Rushneck, consultant for PJB Laboratories. The costs employed were those which PJB was charging EPA-EGD on an IFB contract. For the 12 data sets* associated with the typical verification episode, 36 sample analyses** are performed; 24 of these are solely for data quality (QA) information⁴. The cost for 12 data sets (assuming a cost of \$294 per data sets) without QA would be:

$$12 \times \$294 = \$3,528$$

So the cost for QA is:

$$\$10,584 - \$3,528 = \$7,056$$

By using isotope dilution where recovery can be evaluated from labeled compound data, sample analyses solely for QA are limited to a precision evaluation, i.e., two additional analyses. The cost per analysis, however, can be expected to increase slightly, primarily due to the reporting costs which now are spread over a smaller number of sample analyses. It is estimated that this will cost an additional \$21 per sample. Table 9 summarizes these costs.

Consequently, for 12 sets of data using isotope dilution, 14 sample analyses are performed. Two of these are solely QA information. The cost for 12 data sets was already determined to be \$3,528 without QA, so the cost of QA when using isotope dilution is:

$$\$4,410 - \$3,528 = \$882$$

This corresponds to an 87.5 percent reduction in QA costs compared with the existing QA program.

*One data set represents the listing of compounds and concentrations determined for a given sample point at a given time.

**One sample analysis represents the effort associated with preparing, analyzing and reporting the compounds and concentrations associated with one each base/neutral fraction and volatile fraction.

TABLE 8

COST OF TYPICAL VERIFICATION EPISODE EMPLOYING EXISTING QA PROGRAM

Data Sets	Sample Analyses	Activity	Unit Cost (\$)	Cost (\$)
1	1	Priority pollutant screening to determine background concentrations.	294	294
	6	Single sample precision data on background concentrations; surrogates in all samples.	294	1,764
	6	Single sample recovery data; duplicate samples spiked with priority pollutants at 2, 10, and 100 times the background levels; surrogates in all samples.	294	1,764
2-7	12	Multiple sample precision on background concentrations; each sample run in duplicate; surrogates in all samples.	294	3,528
	6	Multiple sample recovery data; surrogates in all samples.	294	1,764
8-12	5	Surrogate Spiking only.	294	1,470
TOTAL COST				\$10,584

TABLE 9

PROJECTED COST OF TYPICAL VERIFICATION EPISODE EMPLOYING ISOTOPE DILUTION

Data Sets	Sample Analyses	Activity	Unit Cost (\$)	Cost (\$)
1	3	Single sample precision data for isotope dilution data.	294	945
2-12	11	Isotope dilution analysis; QA information for recovery and precision is derived from labeled priority pollutant spikes.	315	3,465
TOTAL COST				\$4,410

SECTION 8

FUNDAMENTAL STUDIES

This section describes the fundamental studies associated with (1) selecting the best two m/z 's for measurement, (2) identifying the best data reduction methods, (3) evaluating isotopic purity, and (4) investigating the effects of instrument variables.

SELECTION OF M/Z 's FOR QUANTITATION

There are three key considerations in selecting a pair of m/z 's for quantitation. They are:

- The isotope ratio relationship between the naturally abundant sample and labeled internal standard,
- Potential chemical interferences at both m/z 's, and
- The relative ion abundance (intensity) of the more abundant m/z 's in sample and internal standard.

The following will discuss these items in detail.

Mathematical study of the propagation of errors indicate that the best two m/z 's at which to quantitate will be those which yield the largest value of R_x/R_y where:

$R_x = a_x/b_x$;	The isotope ratio of the naturally abundant compound X.
$R_y = a_y/b_y$;	The isotope ratio of the labeled compound X.
a_x and a_y are	The abundance at $m/z = a$ for compound X and its labeled analog Y, respectively (see note).
b_x and b_y are	Same as a_x and a_y but for $m/z = b$.

Note: In this report, a 's and b 's are defined such that $R_x > R_y$. Although this is not a mathematical necessity, it is a convenient convention to which to adhere. A complete discussion of this topic is available (see Reference 3).

When m/z's are selected using this criterion, propagation of random and/or systematic errors are minimized and the maximum concentration range for useful application is achieved. Propagation of random errors can be predicted according to the equation given by Colby² as:

$$\frac{d(y/y)}{x/y} = \left[\left(\frac{\partial f(R_x, R_y, R_m)}{\partial R_m} \right)^2 + \left(\frac{\partial f(R_x, R_y, R_m)}{\partial R_x} \right)^2 + \left(\frac{\partial f(R_x, R_y, R_m)}{\partial R_y} \right)^2 \right]^{1/2} \quad (1)$$

where

$$f(R_x, R_y, R_m) = \frac{(R_m - R_y)(R_x + 1)}{(R_x - R_m)(R_y + 1)} \quad (2)$$

After substitution of $f(R_x, R_y, R_m)$ in Equation 1 according to Equation 2 and differentiation, $d(x/y)/(x/y)$ becomes:

$$\frac{d(x/y)}{(x/y)} = \left[\left(\frac{(R_x - R_y)dR_m}{(R_y - R_m)(R_m - R_x)} \right)^2 + \left(\frac{(R_m + 1)dR_x}{(R_x + 1)(R_m - R_x)} \right)^2 + \left(\frac{(R_m + 1)dR_y}{(R_y + 1)(R_y - R_m)} \right)^2 \right]^{1/2} \quad (3)$$

From this, the relative contributions of each of the three terms in Equation 8.1.1 can be evaluated independently. The results are shown graphically in Figure 1 for a hypothetical compound for which $R_x = 100$ and $R_y = 0.01$ and for a measurement error, dR/R , of ± 5 percent for all isotope ratios. It is anticipated that these hypothetical compound values are fairly typical of many analytical situations encountered in the analysis of environmental samples. From the data displayed in Figure 1, it can be seen that contributions to the relative propagated error are small for R_x and R_y compared to those associated with R_m . That is:

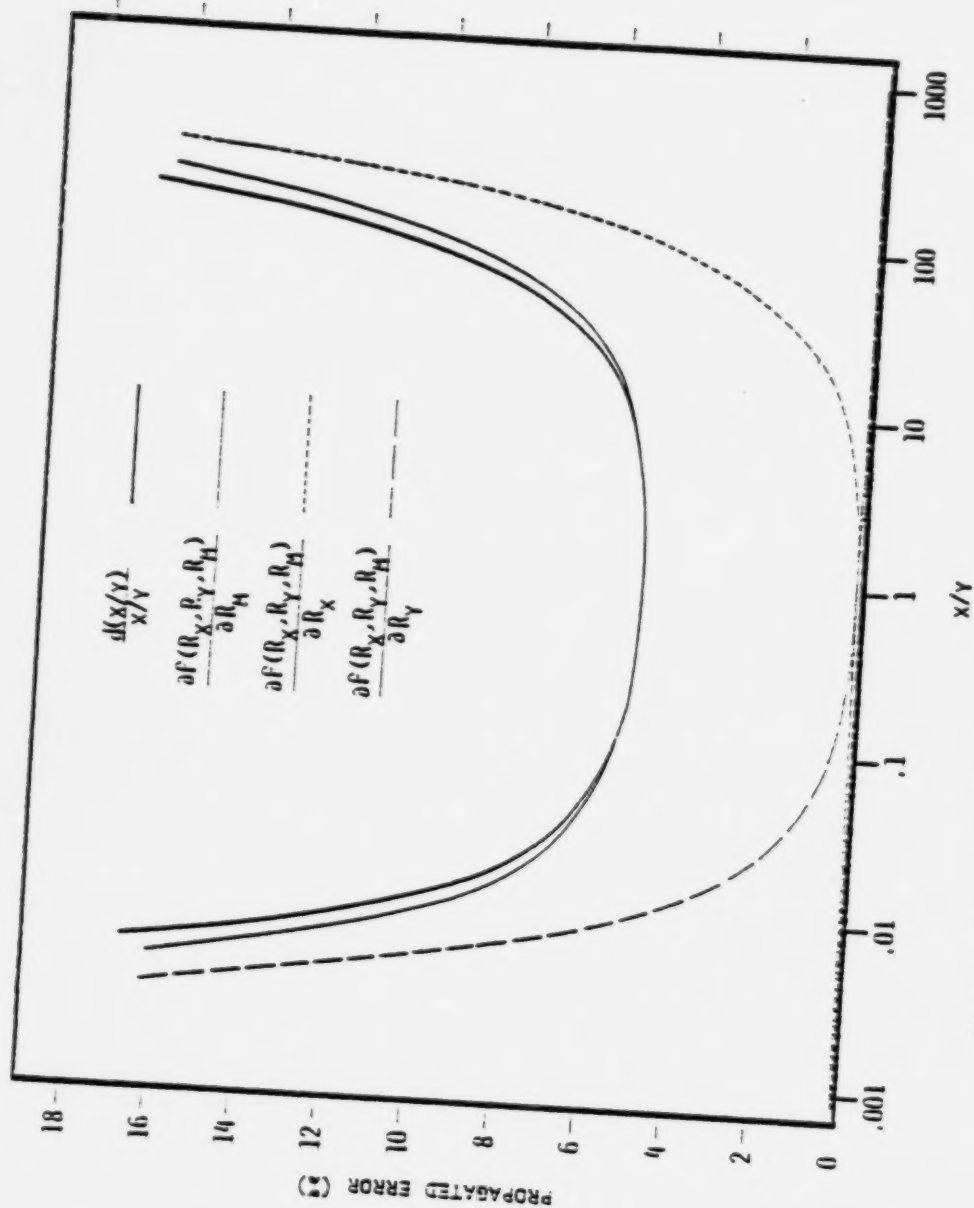


Figure 1. Propagation of Errors in Isotope Dilution GC/MS.

$$\frac{d(x/y)}{(x/y)} = \frac{(R_x - R_y) dR_m}{(R_y - R_m)(R_m - R_x)} \quad (4)$$

If an acceptable analytical error is defined as twice the measurement error in R_m , two limiting values of x/y exist; one for large values of R_m , $(x/y)_{\max}$, and one for small values of R_m , $(x/y)_{\min}$. These are arrived at as follows:

First:

$$\frac{d(x/y)}{(x/y)} = 2 \frac{dR_m}{R_m} = \frac{(R_x - R_y) dR_m}{(R_y - R_m)(R_m - R_x)} \quad (5)$$

or:

$$2 = \frac{(R_x - R_y) R_m}{(R_y - R_m)(R_m - R_x)} \quad (6)$$

For $(x/y)_{\min}$, $R_x \gg R_m$ and $R_x \gg R_y$, so:

$$2 = \frac{R_x R_m}{(R_y - R_m)(-R_x)} = \frac{R_m}{R_m - R_y} \quad (7)$$

On rearrangement this becomes:

$$R_m = 2R_y \quad (8)$$

For $(x/y)_{\max}$, $R_y \gg R_x$ and $R_y \gg R_m$, so:

$$2 = \frac{R_x R_m}{-R_m(R_m - R_x)} = \frac{R_x}{R_x - R_m} \quad (9)$$

On rearrangement this becomes:

$$R_m = \frac{1}{2} R_x \quad (10)$$

Consequently, the analytical result will be acceptable if the condition,

$$2R_y < R_m < \frac{1}{2} R_x \quad (11)$$

is satisfied.

The relationships expressed in Equations 8 and 10 can be used to describe the range of concentrations over which acceptable quantitation can be expected. In terms of orders of magnitude, this is arrived at by determining the difference between $\log(x/y)_{\max}$ and $\log(x/y)_{\min}$. Because it has been shown³ that:

$$\frac{x}{y} = \frac{(R_m - R_y)(R_x + 1)}{(R_x - R_m)(R_y + 1)} \quad (12)$$

it can readily be shown that:

$$\left(\frac{x}{y}\right)_{\max} = \frac{\left(\frac{1}{2} R_x - R_y\right)(R_x + 1)}{\left(R_x - \frac{1}{2} R_x\right)(R_y + 1)} \quad (13)$$

and that:

$$\left(\frac{x}{y}\right)_{\min} = \frac{(2R_y - R_y)(R_x + 1)}{(R_x - 2R_y)(R_y + 1)} \quad (14)$$

For $R_x \gg R_y$, Equations 13 and 14 reduce to

$$\left(\frac{x}{y}\right)_{\max} \cong \frac{R_x + 1}{R_y + 1} \quad (15)$$

$$\left(\frac{x}{y}\right)_{\min} \cong \frac{R_y(R_x + 1)}{R_x(R_y + 1)} \quad (16)$$

Now,

$$\log\left(\frac{x}{y}\right)_{\max} - \log\left(\frac{x}{y}\right)_{\min} = \log \frac{R_x + 1}{R_y + 1} - \log \frac{R_y(R_x + 1)}{R_x(R_y + 1)} = \log \frac{R_x}{R_y} \quad (17)$$

Consequently, to maximize the concentration range of acceptable application, the value of $\log(R_x/R_y)$ or R_x/R_y must be maximized. For the hypothetical case data plotted in Figure 1, the anticipated concentration range for acceptable application would be:

$$\log \frac{100}{.01} = 4$$

that is, four orders of magnitude in concentration.

In practice, R_x/R_y can be maximized in two ways: through selection of m/z 's for quantitation and through increased isotopic purity of the labeled internal standard. Selecting m/z 's, however, is always the more cost-effective approach to attempt first. A convenient technique has been developed to eliminate direct calculation of all potentially useful R_x/R_y values through the use of interference abundance ratios (r_i) where:

$$r_i = \frac{\text{Measured Abundance at } m/z_i \text{ for Naturally Abundant Compound}}{\text{Measured Abundance at } m/z_i \text{ for Labeled Compound}}$$

Because

$$\frac{R_x}{R_y} = \frac{a_x/b_x}{a_y/b_y} = \frac{a_x}{a_y} \frac{b_y}{b_x} = r_a r_b^{-1} \quad (18)$$

and because

$$R_x > R_y \quad (19)$$

R_x/R_y can be maximized by maximizing a_x/a_y and minimizing b_x/b_y . Consequently, a compilation similar to that shown in Table 10 can be used to choose m/z 's. The largest r_i value becomes $m/z = a$ and the smallest, $m/z = b$.

To evaluate R_x/R_y maximization as a means of selecting m/z for quantitation, analyses were performed to measure benzene, toluene, and 1,2-dichloroethane using benzene- d_6 , toluene- d_8 , and 1,2-dichloroethane- d_4 , respectively, as internal standards.

Samples were prepared from laboratory pure water by spiking with known quantities of these compounds. To determine R_x and R_y , samples containing only unlabeled or labeled compounds, respectively, were analyzed by purge and trap GC/MS. Tables equivalent to Table 10 were prepared for each of the four compounds and those m/z 's yielding the largest and smallest abundance ratios assigned as $m/z = a$ and $m/z = b$, respectively. The ability to quantitate at those m/z 's was estimated from deviations between the data points and a logarithmically weighted linear regression line through those data points. Samples analyzed had unlabeled compound concentrations of 1, 3, 10, 30, 100, 300, and 1000 $\mu\text{g/l}$ and labeled compound concentrations of 30 $\mu\text{g/l}$. The results are shown in Table 11

TABLE 10
INTERREFERENCE ABUNDANCE RATIO TABLE

m/z		1	2	3	4		i
Abundance at m/z _j	x						
	y						
Abundance Ratio		r ₁	r ₂	r ₃	r ₄		r _i

TABLE 11
EFFECTS OF $\log(R_x/R_y)$ MAXIMIZATION ON QUANTITATION

Compound	m/z's	$\log(R_x/R_y)$	Correlation Coefficient	Mean Deviation From Regression Line (%)
Benzene	78/84	5.07	.998	9
	50/56	4.99	.994	23
	77/82	4.81	.981	36
Toluene	92/100	8.84	.999	10
	91/98	8.16	.998	12
1,2-Dichloroethane	62/67	9.34	.998	9
	64/67	8.84	.997	11
	98/104	7.55	.993	18
	49/53	6.73	.997	16
	98/102	4.00	.975	36

for the more abundant m/z's in the acquired spectra. For the most part, there is good agreement between the ranking of m/z pairs based on the $\log(R_x/R_y)$ values for a given compound. For benzene, however, the minor differences in $\log(R_x/R_y)$ are accompanied by major differences in the mean deviation from regression line values. This is apparently due to the fact that the $\log(R_x/R_y)$ does not take into account the effect of absolute ion abundance. Clearly, a more abundant m/z will yield a measurement of greater precision (and presumably accuracy) than a less abundant m/z.

As has been pointed out, absolute ion abundance has an effect on the ability to perform isotope ratio measurements. When low level ion signals are encountered and ion counting can be employed, the precision of the measurements is given by the square root of the number of counts. For typical GC/MS data, however, ion counting is not practical and measurement precision becomes an experimentally determined entity. Because of this experimental character, it was unreasonable to develop criteria for m/z selection from basic principles. However, it was possible to develop an empirical parameter based on absolute abundance. For the purpose of this presentation, this parameter will be referred to as the Abundance Parameter (AP). It is given by:

$$AP = \log \frac{2}{a_x + b_y} \quad (20)$$

where a_x and b_y are abundances expressed as fraction of total ionization for isotopes a and b determined for pure compounds x and y. When a_x and b_y are large, AP becomes small and vice versa. By subtracting AP from $\log(R_x/R_y)$, a correction to this previously developed m/z selection parameter is produced. When $a_x = b_y = 1$, the ideal two-isotope situation, $AP = 0$ and no correction to $\log(R_x/R_y)$ is made.

To evaluate the appropriateness of the AP in m/z selection, the data used to generate Table 11 was reevaluated and AP's calculated. The results, shown in Table 12, indicate that the AP is useful in m/z selection. This is particularly true when the $\log(R_x/R_y)$ values are similar as they were for three sets of m/z's investigated for benzene.

CALCULATION METHODS

There are four fundamentally related but distinct methods available for quantitative calculations. These are:

$$\frac{x}{y} = R_m \quad (21)$$

TABLE 12
EFFECT OF ABUNDANCE ON M/Z SELECTION

Compound	m/z's	$\log\left(\frac{2}{a_x + b_y}\right)$	$\log\left(\frac{R_x}{R_y}\right)$	$\log\left(\frac{R_x}{R_y}\right) - \log\left(\frac{2}{a_x + b_y}\right)$	Correlation Coefficient	From Regression Line (%)
Benzene	78/84	0.285	5.07	4.79	.998	9
	50/56	1.08	4.99	3.91	.994	23
	77/82	1.02	4.81	3.79	.981	36
Toluene	92/100	0.547	8.84	8.29	.999	10
	91/98	0.441	8.16	7.72	.998	12
1,2-Dichloroethane	62/67	0.399	9.34	8.94	.998	9
	64/67	0.921	8.84	7.92	.997	11
	98/104	1.36	7.55	6.19	.993	18
	49/53	0.996	6.73	5.73	.997	16
	98/102	1.28	4.00	2.73	.975	36

$$\frac{x}{y} = \frac{R_m(R_x + 1)}{R_y + 1} \quad (22)$$

$$\frac{x}{y} = \frac{R_m - R_y}{R_y + 1} \quad (23)$$

$$\frac{x}{y} = \frac{(R_y - R_m)(R_x + 1)}{(R_m - R_x)(R_y + 1)} \quad (24)$$

The assumptions involved in each are associated with extents to which R_m approximates R_x and/or R_y . When

$$R_y \ll R_m \ll R_x$$

Equation 21 can be expected to yield a simple linear curve. However, if $R \rightarrow R_x$ or R_y , Equations 22 or 23, respectively, must be employed in order to provide the linear relation. When R_m can approach both R_x and R_y , Equation 24 becomes necessary if linearity is to be expected. Naturally, when corrections for R_x and/or R_y are required, the propagation of errors must be considered. This propagated error, however, should be considered in light of the potential for introducing systematic errors (nonlinearity) associated with the assumptions already mentioned.

Because these quantitative relationships consist of a trade-off involving an increase of propagated error in order to reduce systematic error, it is appropriate to identify the circumstances under which each method is applicable. It has already been shown that the propagated errors for Equation 24 will be acceptable for R_m 's where $2R_y < R_m < .5R_x$. When the same magnitude of error is applied to systematic error, it can be shown that the equation involving the maximum potential for systematic error,

$$\frac{x}{y} = R_m \quad (25)$$

will yield acceptable results for R_m 's where $10 R_y < R_m < 1R_x$. This is done as follows:

First determine R_m in terms of R_x for $(x/y)_{\max}$ where:

$$\left(\frac{x}{y}\right)_{\max} = 1.1R_m = \frac{(R_y - R_m)(R_x + 1)}{(R_m - R_x)(R_y + 1)} \quad (26)$$

When Equation 26 is simplified using approximations appropriate for $R_x \gg 1$, $R_y \ll R_m$ and $R_y \gg 0$, the result becomes

$$1.1 R_m = \frac{-R_m R_x}{R_m - R_x} \quad (27)$$

or

$$R_m = 0.1 R_x \quad (28)$$

Similarly, for $(x/y)_{\min}$

$$\left(\frac{x}{y}\right)_{\min} = 0.9 R_m = \frac{(R_y - R_m)(R_x + 1)}{(R_m - R_x)(R_y + 1)} \quad (29)$$

On condensation using approximations appropriate for $R_y \ll 1$, $R_m \ll R_x$ and $R_x \gg$, the result value for R_m in terms of R_y becomes:

$$0.9 R_m = R_m - R_y \quad (30)$$

or

$$R_m = 10 R_y \quad (31)$$

This more restricted R_m range results in approximately a two order of magnitude reduction in the concentration range for acceptable application compared to that encountered with Equation 24. This can be seen graphically in Figure 2. Similar calculations can be done for the other two methods of calculation. A summary of all four methods is given in Table 13. To select an appropriate equation for determining the concentration ratio of sample to internal standard, it is only necessary to evaluate the relative magnitude of R_m with respect to R_x and/or R_y and choose the proper equation from those listed in Table 13.

In order to evaluate these predictions, data for the compounds described in Section 1 were evaluated using each of the four equations (1 through 4). The results, given in Table 14, seem to indicate that calculation method per se is not a significant factor in data treatment. This conclusion, however, must be considered in light of the $\log(R_x/R_y)$ values associated with the data; all are relatively large, i.e., four or greater, when compared with the log of the concentration range evaluated

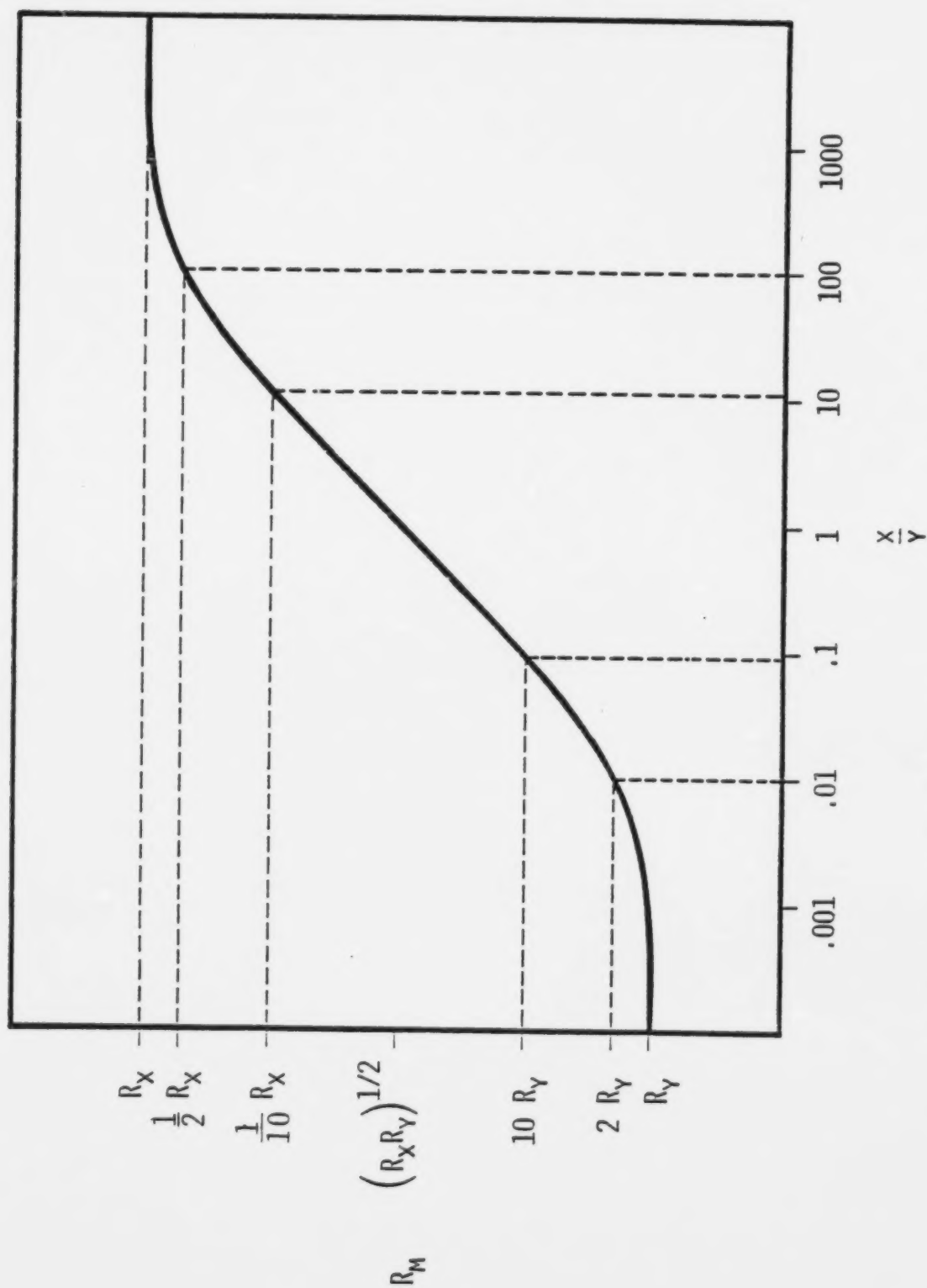


Figure 2. Illustration of Concentration Range of Acceptable Application

TABLE 13

 R_m RESTRICTIONS FOR ACCEPTABLE APPLICATION

Equation	R_m Restriction
$\frac{x}{y} = R_m$	$10R_y < R_m < .1R_x$
$\frac{x}{y} = \frac{R_m(R_x + 1)}{R_x - R_m}$	$10R_y < R_m < .5R_x$
$\frac{x}{y} = \frac{R_m - R_y}{R_y + 1}$	$2R_y < R_m < .1R_x$
$\frac{x}{y} = \frac{(R_y - R_m)(R_x + 1)}{(R_m - R_y)(R_y + 1)}$	$2R_y < R_m < .5R_x$

TABLE 14
EFFECTS OF DATA REDUCTION METHOD ON QUANTITATION

Compound	m/z's	$\log(R_x/R_y)$	Deviation From Regression Line (%)			
			Equation			
			8.2.1	8.2.2	8.2.3	8.2.4
Benzene	78/84	5.07	12	10	10	9
	50/56	4.99	10	10	28	23
	77/82	4.81	39	37	40	36
Toluene	92/100	8.84	7	10	10	10
	91/98	8.16	9	9	12	12
1,2-Dichloroethane	62/67	9.34	9	10	10	9
	64/67	8.84	14	14	14	11
	98/104	7.55	28	28	28	18
	49/53	6.73	11	11	11	16
	98/102	4.00	54	69	69	36
Mean			19.3	20.8	23.2	18.0
C.V.			0.82	0.93	0.83	0.58

(see Equation 17). Pursuing the evaluation with a wider range of concentrations, although theoretically possible, would be of little real value because the concentration range covered was limited at one end by detection limit and at the other by ion source saturation. Consequently, it seems unlikely that much more than three orders of magnitude in concentration need be investigated. In light of the facts that (1) equivalent or better results are achieved using Equation 4 and (2) a wider applicable concentration range is available when needed using Equation 24, it is logical to always recommend Equation 24 for routine applications.

LABEL PURITY

Although isotopic purity of commercially available compounds is generally advertised to be 98 percent or greater, it is possible that material of lower purity may be encountered. In order to estimate the magnitude of these potential errors, the propagated systematic error incorporated into three hypothetical analytical situations was evaluated for a five percent error in the isotopic purity (R_y). The results are plotted in Figure 3.

As can be seen in Figure 3, the direction of the error induces an apparent concentration larger than the true value. It should be pointed out, however, that the error rapidly becomes small at higher sample concentrations and consequently would have only a small effect on quantitation throughout most of the concentration range. Also, the error will be eliminated if R_y is determined for each lot of labeled compound and employed in the calculations as described in "CALCULATION METHODS".

INSTRUMENT VARIABLES

It was anticipated that instrument variables would have less effect on isotope dilution quantitation than they would on conventional GC/MS quantitation. Short-term variations in ion source temperature and pressure, and emission current are known to alter the relative abundance of ion fragments and consequently, quantitation values would be effected. This is not the case with isotope ratios which are insensitive to these parameters. In the long term, and particularly when an instrument is retuned, changes in relative abundance patterns due to changes in ion transmission can be major if care is not taken. To help overcome this problem, analysis of abundance standards, such as perfluorotributylamine (FC43), bis(pentafluorophenyl)-phenylphosphine⁵ (DFTPP)⁵ or bromofluoro-benzene (BFB)¹ are included as part of tuning procedures. Isotope ratios, however, are relatively unaffected by this part of the tuning procedures because the m/z 's involved are separated by only a few m/z 's.

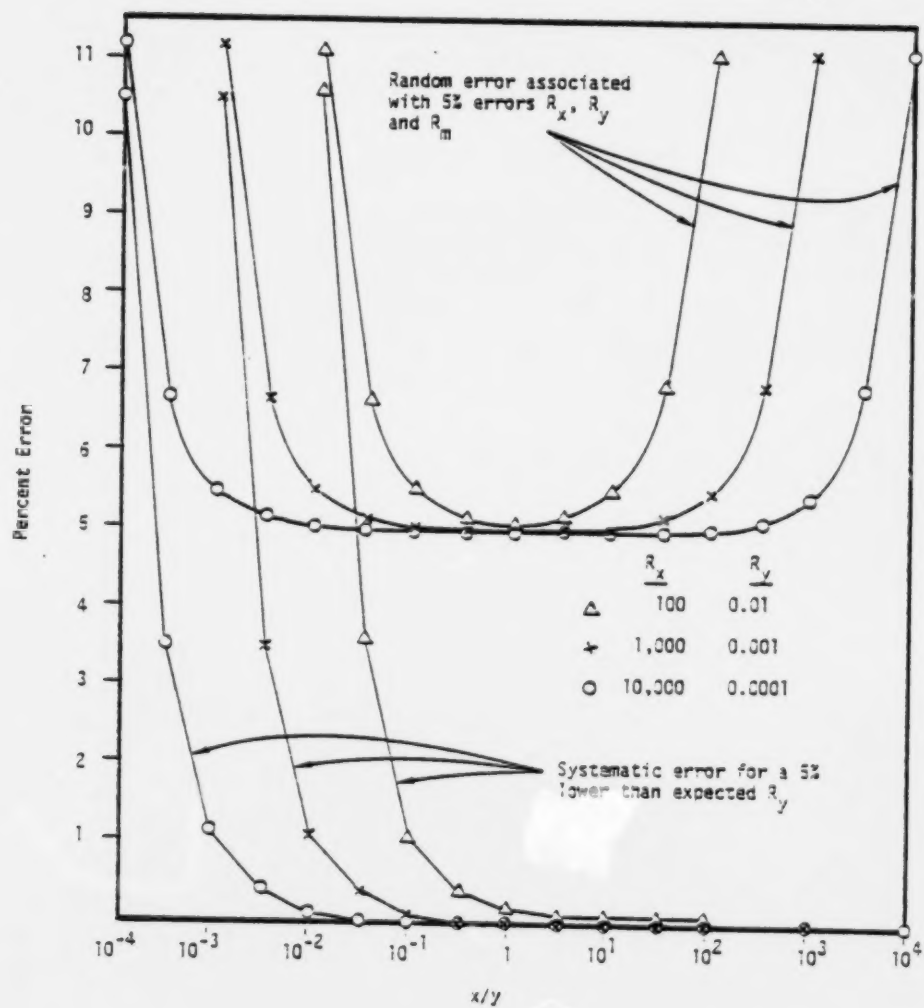


Figure 3. Predicted Error in x/y Based on a Five Percent Error in Label Purity.

To test this hypothesis, 37 priority pollutants which are determined in the base/neutral fraction, were injected into the GC/MS while it was tuned for satisfactory and unsatisfactory DFTPP abundance patterns (Figures 4 and 5, and Table 15 and 16), changes in relative response and isotope ratios were determined. For relative responses, there was a change 43 percent on the average compared with a six percent change on the average for isotope ratios. The six percent change is equivalent to the isotope ratio measurement precision for replicate samples analyzed under constant conditions using the same GC/MS as was used for this test. That is, no detectable change in isotope ratios was observed. For relative responses measured under constant conditions, precision is about ten percent. Consequently, a significant dependence on abundance tuning was observed for relative responses but not for isotope ratios. It should be pointed out that although the DFTPP abundance pattern was altered during this test, mass resolution was maintained. This is because any attempts to use a mass spectrometer at less than unit mass resolution can be expected to fail.

Even though abundance tuning does not affect isotope ratios and consequently would not affect the accuracy of isotope dilution quantitation, this type of tuning is judged highly desirable. The reason for this is based on the need to evaluate the acquired data for compound identifications prior to quantitation. Reliable and reproducible abundance patterns are essential to the success of this process.

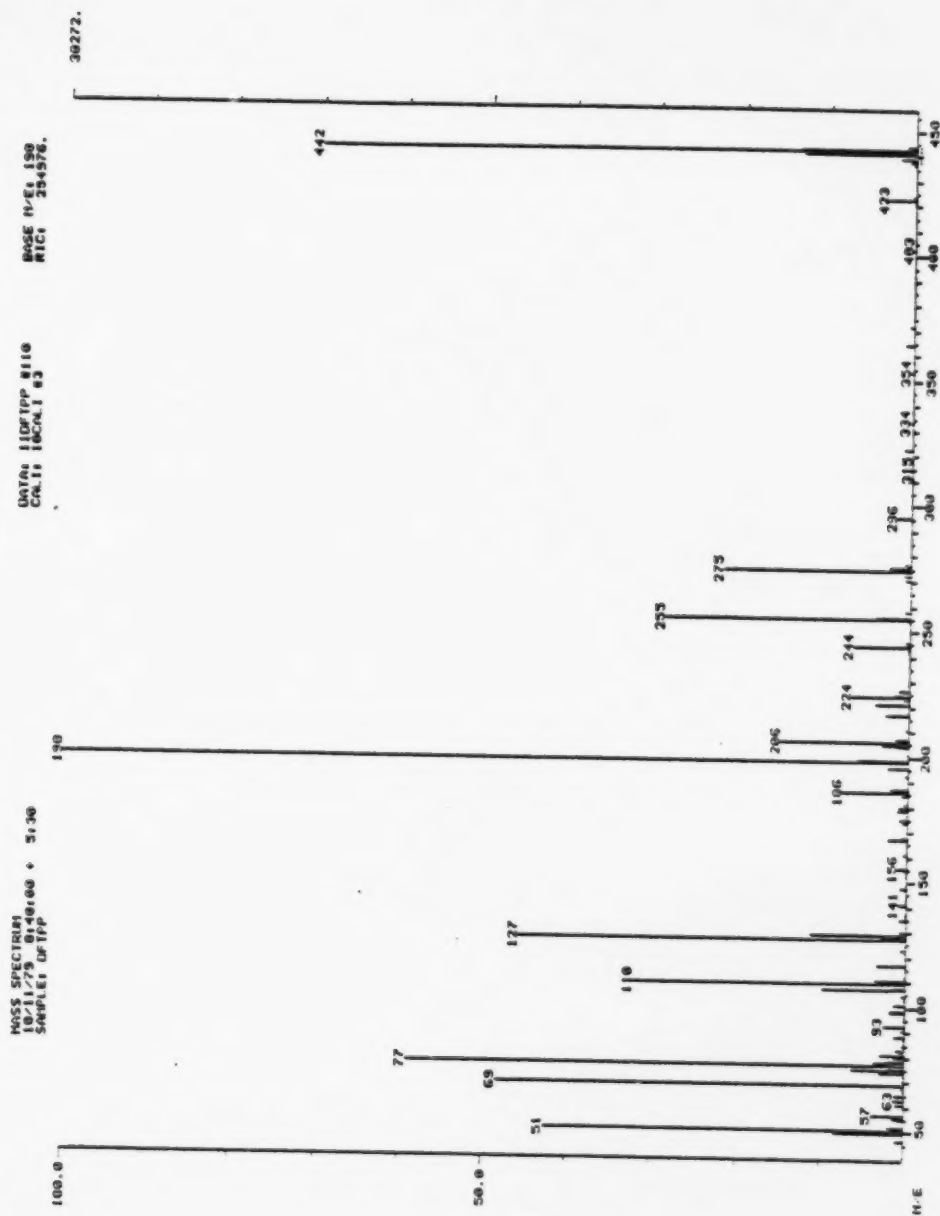


Figure 4. Good DFTPP Spectrum

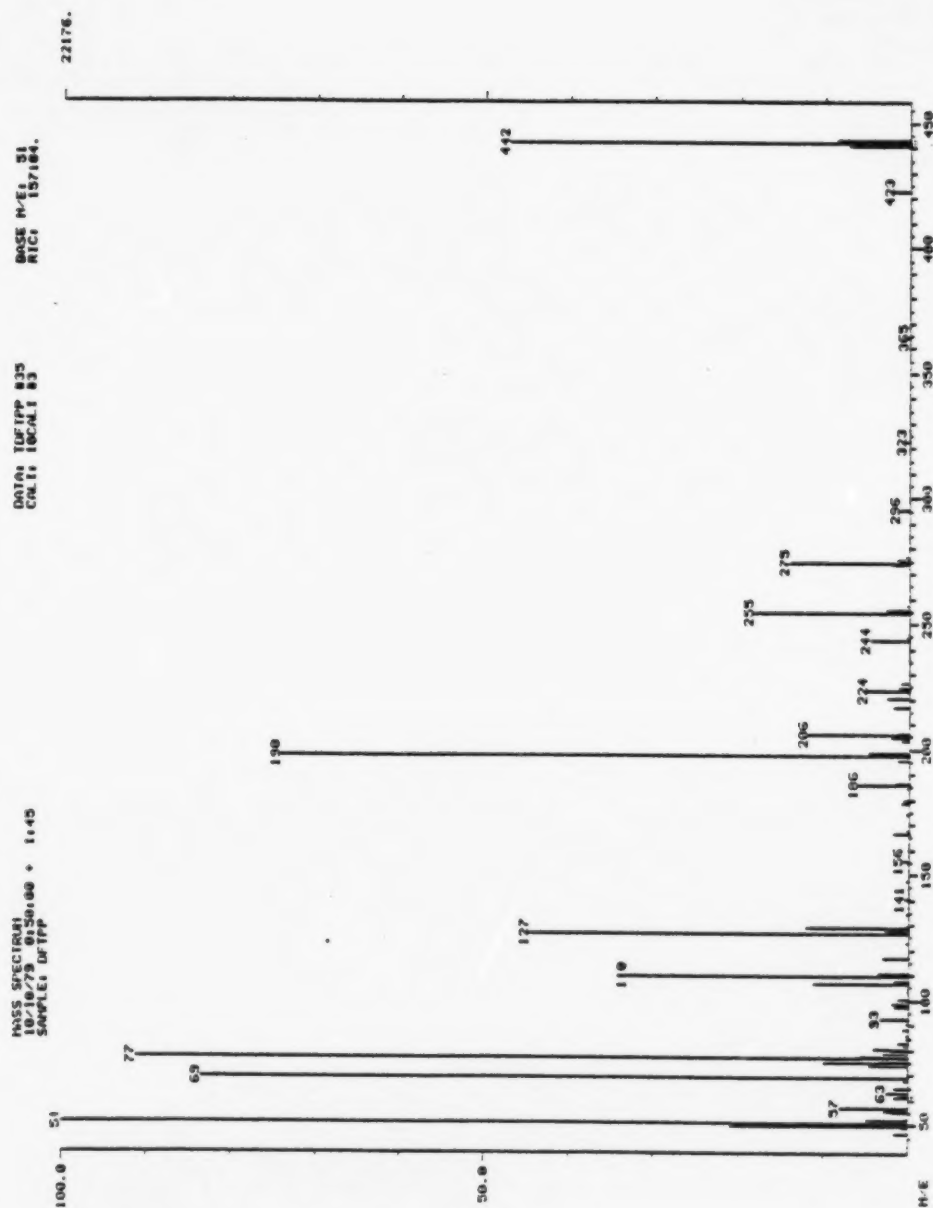


Figure 5. Bad DFTPP Spectrum

TABLE 15
GOOD DFTPP SPECTRUM

MASS LIST DATA: 110DFTPP # 110 BASE M/E: 198
10/11/79 8:40:00 + 3:30 CALI: LOCALI # 3 RIC: 234976.
SAMPLE: DFTPP

47	0.30	MINIMA	HIN	INTEN:	191.
444 #	0	MAXIMA			
MASS	% RA	MASS	% RA		
47	0.96	199	6.03		
50	8.35	204	1.21		
51	42.56	205	3.20		
52	1.90	206	13.13		
55	0.71	207	1.63		
56	1.29	217	2.70		
57	3.84	221	4.00		
61	0.90	223	0.38		
63	1.27	224	6.82		
65	1.01	225	1.84		
69	48.41	227	1.25		
74	2.96	244	8.63		
75	6.12	245	0.61		
76	2.04	255	28.89		
77	59.11	256	4.13		
78	3.61	258	0.57		
79	2.15	271	0.55		
80	1.30	273	0.72		
81	2.89	275	22.12		
82	0.33	276	2.52		
83	1.12	277	0.73		
89	1.13	296	1.65		
93	2.56	323	0.98		
98	1.78	365	0.96		
99	1.77	423	3.21		
101	1.34	438	0.75		
107	9.88	439	1.78		
108	1.56	441	13.29		
110	32.11	442	70.15		
111	3.67	443	13.67		
117	3.38	444	1.01		
123	0.35				
127	45.74				
128	3.09				
129	11.27				
130	0.71				
135	0.80				
141	0.91				
148	0.75				
154	1.01				
167	2.24				
168	0.69				
174	0.60				
175	0.92				
179	1.26				
180	1.00				
185	0.69				
186	7.65				
187	2.11				
196	2.44				
198	100.00				

TABLE 16
BAD DFTPP SPECTRUM

MASS LIST DATA: TDFTPP # 30 BASE N/E: 31
10/10/79 8:30:00 + 1:45 CALI: LOCALI # 3 RIC: 157184
SAMPLE: DFTPP

47	0.50	MINIMA	MIN INTEN:	111.
444 #	0	MAXIMA		
MASS	I	RA	MASS	I
				RA
47	1.71		221	2.60
49	0.80		224	4.87
50	21.07		225	1.20
51	100.00		227	0.84
52	5.05		244	4.50
55	2.01		255	18.27
56	2.94		256	2.78
57	7.97		274	1.96
61	1.70		275	13.98
62	1.01		276	1.52
63	2.60		296	1.12
65	1.69		423	1.85
68	0.61		441	7.31
69	83.55		442	47.11
74	4.73		443	8.65
75	9.90			
76	3.48			
77	91.20			
78	5.69			
79	3.00			
80	2.25			
81	4.12			
82	0.58			
83	1.14			
89	0.60			
93	3.33			
98	1.65			
99	1.89			
101	1.28			
107	11.22			
108	1.78			
110	33.13			
111	3.69			
117	3.03			
127	44.59			
128	2.93			
129	12.16			
156	0.58			
167	1.75			
179	0.97			
180	0.62			
186	5.88			
187	1.37			
196	1.36			
198	74.03			
199	4.78			
204	0.91			
205	2.11			
206	11.76			
208	1.29			
217	1.81			

SECTION 9

WASTE WATER DEMONSTRATIONS

MUNICIPAL WASTE WATER ANALYSES

Because the anticipated costs associated with performing priority pollutant analyses using isotope dilution methodology were favorable, two waste waters associated with publically owned treatment works (POTW's), i.e., municipal waste water treatment facilities, and an organic-free laboratory water were analyzed. One POTW sample was an influent to the facility and the other was the corresponding treated effluent.

Volatile organic compounds were analyzed using the conventional purge-and-trap GC/MS techniques after spiking the 5-ml sample aliquot with approximately 150 ng each of the available labeled compounds. The samples were analyzed as received plus spiked at levels of 100 and 1000 $\mu\text{g/l}$ with priority pollutants. The 100 $\mu\text{g/l}$ spiked lab water sample was prepared twice and one of the data sets was used to calibrate labeled compound concentrations. Base/neutral and acid extractable compounds were also prepared in the conventional manner. Each 1-liter sample was prepared as received as well as spiked with 100 and 1000 $\mu\text{g/l}$ quantities of priority pollutants. In addition, each sample was spiked with 100 μg each of the available labeled priority pollutants.

Concentrations, in $\mu\text{g/l}$, for the test compounds (x's) were calculated using isotope dilution according to the equation:

$$x = y'R_m \quad (32)$$

where y' is the apparent concentration of labeled compound as calculated from the 100 $\mu\text{g/l}$ calibration analysis and R_m is the isotope ratio of the mixture for the selected m/z 's. In all test cases, R_m fell well in the window defined by $10 R_y$ to $0.1 R_x$ (see Table 13). Any initial priority pollutant concentrations in unspiked samples were subtracted from the values calculated for the spiked samples. The percent error was then determined for each spiking level. The results are shown in Table 17.

Concentrations were also calculated using the method of standard additions. Any initial priority pollutant concentrations in the unspiked samples were subtracted from the value determined for the 100 $\mu\text{g/l}$

TABLE 17
APPLICATION OF ISOTOPE DILUTION TO POTW SAMPLES

Compound	Percent Error*					
	Standard Additions			Isotope Dilution		
	Lab Water	POTW Influent	POTW Effluent	Lab Water	POTW Influent	POTW Effluent
Benzene	11	11	11	14	4	9
Toluene	31	31	44	17	6	11
Methylene Chloride	39	38	52	13	11	8
Phenol	133	212	**	7	8	4
1,2-Dichlorobenzene	71	52	42	23	21	22
Naphthalene	0	18	47	26	41	27
Nitrobenzene	2010	6	34	26	5	18
Chrysene	49	81	23	9	14	7
Benzo(a)pyrene	42	72	11	14	24	7
Mean	47 [†]	56	33	17	15	13

*Because standard addition analyses require two sample preparations and measurements per determination and isotope dilution requires only one per determination, the percent error values for standard additions are based on one calculated concentration while the isotope dilution values are based on two calculated concentrations.

**Undefined; negative concentration.

†Nitrobenzene value not included in calculation.

samples. Percent errors were determined only for the 100 $\mu\text{g/l}$ spiked levels because two sample analyses are required for each determined concentration. The results are shown in Table 17. Because each value determined by the method of standard additions required two sample

preparations and two GC/MS analyses, it can be inferred that the costs to analyze samples by this method will be twice that of samples analyzed by isotope dilution.

On the average, isotope dilution analysis of the POTW samples resulted in a 15 percent error compared with a 45 percent error using standard additions.

SIMULATED WASTE WATER ANALYSES

Data previously acquired during an investigation of samples which foamed during purge-and-trap analysis were reinvestigated. The samples analyzed were laboratory pure water, laboratory pure water spiked with dishwashing detergent (Purex Balsum Trend) and laboratory pure water spiked with dishwashing detergent and a silicone antifoaming agent. Only the volatile organic fraction was investigated in this study. The sample preparation and data reduction were equivalent to those described previously for Municipal Waste Water, except that priority pollutants were spiked at 1, 3, 10, 30, 100, 300, and 1000 $\mu\text{g/l}$. A comparison of the data reduction methods is given in Table 18. Again, it can be seen that isotope dilution determinations result in significantly lower percent errors than do data generated by the method of standard additions. On the average, isotope dilution resulted in a 28 percent error compared with a 63 percent error for standard additions.

INDUSTRIAL WASTE WATERS ANALYSES

Because both the POTW and simulated waste water analyses yielded positive results in terms of accuracy when compared with the standard additions method, an expansion of isotope dilution analysis applications was undertaken to include more priority pollutants and additional types of waste waters. This series of experiments involved 10 samples analyzed by S-CUBED and 10 by TRW. Spiked and unspiked aliquots of the samples were analyzed using Methods 624 and 625¹ and using isotope dilution methodology. Details of this experiment can be found in Appendix A of this report. Subsequent to analyzing the samples, the accuracy expressed in terms of percent recovery and the precision of Methods 624 and 625 were compared with those obtained using isotope dilution. Fourteen priority pollutants were analyzed in the base/neutral fractions, eight in the acid fractions and thirteen in the volatile fraction. The mean percent recoveries and mean precisions for these determinations are shown in Table 19.

The most important observations of this study were that the precision and accuracy of calculated percent recoveries determined using isotope

TABLE 18

APPLICATION ON ISOTOPE DILUTION TO SOAP SOLUTION ANALYSIS

Sample	Compound (Data Points)	Mean % Error	
		Standard Addition	Isotope Dilution
Laboratory Pure Water	Benzene (5)	31	27
	Toluene (6)	21	23
	1,2-Dichloroethane (4)	51	17
Soap Solution (Heat)	Benzene (5)	62	23
	Toluene (5)	87	17
	1,2-Dichloroethane (4)	92	26
Soap Solution (Surfactant)	Benzene (5)	59	24
	Toluene (4)	84	44
	1,2-Dichloroethane (4)	108	58

TABLE 19
MEAN VALUE FOR TWENTY INDUSTRIES

Fraction	Mean Calculated Percent Recovery		Mean % SD	
	624/5 ⁽¹⁾	ID ⁽²⁾	624/5	ID
Base/Neutral	56.3	100.0	57.3	12.1
Acid	51.6	99.1	67.5	19.0
Volatile	122.6	98.5	39.5	9.2

(1) EPA Methods 624 and 625

(2) Isotope Dilution

dilution are uniformly unaffected by sample matrix as long as sufficient material is recovered for measurement. This was not found to be the case with more conventional GC/MS methods (as EPA Methods 624 and 625). Also highly significant was the demonstration that large numbers of compounds can be identified and quantitated in environmental samples using isotope dilution methodology in a routine manner with full spectrum data and with readily available instrumentation. A more detailed description of these experiments and their results can be found in Appendix A of this report.

SECTION 10

ISOTOPE DILUTION ANALYSIS PROTOCOLS

Because of the highly favorable results generated in the three application studies involving isotope dilution GC/MS quantitation, analysis protocols for that methodology were prepared for the determination of priority pollutants in water and waste water. The details of these tentative EPA methods can be found in Appendices B and C of this report.

SECTION 11

REFERENCES

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APPENDIX A

ISOTOPE DILUTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE DETERMINATION OF PRIORITY POLLUTANTS IN INDUSTRIAL EFFLUENTS

BRIEF

Isotope dilution GC/MS was investigated for applicability to multicomponent analysis of aqueous industrial effluents and found to be more accurate and more precise than conventional GC/MS methods.

ABSTRACT

Twenty industrial effluents were spiked with thirty-six priority pollutants each and analyzed using isotope dilution and conventional internal standard GC/MS techniques. Using full spectrum scanning data, multicomponent isotope dilution was found to be a readily workable approach for quantitation. It was found to be approximately four times more precise than the conventional internal standard approach and its accuracy was unaffected by sample source.

INTRODUCTION

Stable isotopically labeled materials are being used with increasing frequency as internal standards for quantitative mass spectrometry. The technique involves perturbing the normal isotopic composition of the sample by adding a known quantity of an isotopically enriched analog of the material being determined. The isotope ratio of the resulting mixture falls between that of the original sample and that of the enriched material and is a measure of the concentration ratio of the naturally abundant and enriched materials. The basic principles of isotope dilution are well agreed upon [1,2]. A variety of studies have been reported which identify mechanisms to optimize data acquisition variables [3,4] and data reduction approaches [5,6]. Mathematical models of isotope dilution have been found to agree well with acquired data [6,7] and have been used to develop measurement parameter selection criteria [8].

The majority of quantitative isotope dilution applications have been associated with determination of elements [9,10] or of single organic compounds [11,12]. Organic compounds have generally been determined using selected ion monitoring gas chromatography-mass spectrometry [13,14] to measure isotope ratios, and with a few exceptions, applications have been in areas associated with biological media. Ingram, et al. [15], however, have measured pentachlorophenol (PCP) in industrial effluents using PCP-¹⁸O as a reference compound and a method for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), using TCDD-³⁷Cl as a reference, has been proposed by the U. S. Environmental Protection Agency (EPA) for the monitoring requirements associated with the National Pollutant Discharge Elimination System (NPDES)[16].

Although no specific statements have been made, it appears that one of the major shortcomings in the application of isotope dilution

GC/MS to environmental samples has been an inability to quantitate a sufficient number of compounds in single GC/MS run to justify the analysis expense. This inability has been caused by two factors; (1) there has not been a well-defined list of specific compounds of interest, and (2) only a relatively small number of labeled compounds of environmental interest have been available. As a result of the Clean Water Act passed by Congress in 1977, however, a list of 114 specific toxic organic priority pollutants was established and many of those are now commercially available as stable isotopically labeled forms. Reports of multiple compound determinations, however, have remained extremely limited. Summons, et al. [17], have reported the determination of 12 amino acids using a selected ion monitoring technique and a determination of four volatile compounds in water samples has been described using a full spectrum scanning technique [18]. The purpose of this report is to demonstrate the applicability of multicomponent isotope dilution GC/MS for the determination of priority pollutants and to compare the analytical results with those obtained using more conventional quantitative GC/MS methods, i.e., Methods 624 and 625, which have been proposed by EPA for regulation monitoring [19].

EXPERIMENTAL

Apparatus

Combined gas chromatography/mass spectrometry (GC/MS) was carried out on five separate instruments; these are described in Table I. In addition to the GC/MS equipment, two TEKMAR Model LSC-1 purge and trap devices, one at Systems, Science and Software (S³) and the other at TRW, were employed for the volatile organic compound analysis. The volatile compounds were separated on an eight-foot-long by two-millimeter ID glass column packed with 0.2 percent Carbowax 1500 on 60/80 mesh Caropak C. Compounds in the Base/Neutral Fraction were separated on a five-foot-long by two-millimeter ID glass column packed with three percent SP-225008 on 100/120 mesh Supelcoport and compounds in the Acid Fraction were separated on a glass column with the same dimensions packed with one percent SP-1240DA on 100/120 mesh Supelcoport.

Reagents

Unlabeled priority pollutant compounds, Table II, provided by the U. S. Environmental Protection Agency were used as received to prepare three master spiking solutions. The Base/Neutral spiking solution was prepared in methylene chloride containing ten percent by volume benzene. The Acid spiking solution was prepared using methylene chloride as a solvent and the Volatile compound spiking solution, using methanol. The Base/Neutral and Acid spiking solutions contained 200 µg/mL and the Volatile spiking solutions 20 µg/mL of each of the compounds investigated. The only exceptions to this were 2,4-dichlorophenol which was 240 µg/mL and acrylonitrile which was 100 µg/mL. Labeled compounds were obtained from Aldrich Chemical (Milwaukee, WI), KOR (Cambridge, MA), and Merck, Sharp and Dohme (Pointe Claire-Dorval, Quebec, Canada). Master spiking solutions of the labeled compounds were prepared at the same concentrations and using the same solvent.

systems as unlabeled master spiking solutions. A description of the source and type of labeling is given in Table II. All solvents were obtained from Burdick-Jackson (Muskegon, MI) and were used as received.

Twenty treated wastewater effluents (Table III) were selected as sample matrices for this study. Effluents which were obtained from the same industrial type were selected such that each represented a different manufacturing or wastewater treatment process. For example, one of the coal mining samples came from an acid mine and the other from a non-acid mine. The organic chemical manufacturing effluents were taken from processes involving polymers, fibers and plastics, resins, molding compounds, and solvents. Consequently, each sample represented a different matrix. A more detailed discussion of the samples is not possible due to legal constraints.

Procedure

Four sets of aliquots of each of the 20 water samples were prepared and analyzed. The first two were analyzed according to GC/MS methods which have been proposed for NPDES permit regulation monitoring [19] and the second two, according to the isotope dilution procedure described below. Aliquot No. 1 was prepared and analyzed as received; Aliquot No. 2 was spiked with unlabeled priority pollutants; Aliquot No. 3, with labeled priority pollutants and Aliquot No. 4 with both labeled and unlabeled priority pollutants. After spiking, the solutions were allowed to equilibrate for a minimum of one hour prior to extraction or purging.

The Base/Neutral and Acid fractions were prepared by extracting the water samples with methylene chloride. A one-liter aliquot was first made basic ($\text{pH} > 11$) by adding 6N NaOH and extracted for 24 hours in a continuous liquid-liquid extractor. (Details describing the extractors are available on request.) The collector containing the

Base/Neutral fraction was then removed, the water sample acidified (pH < 2) and reextracted for 24 hours to produce the Acid fraction. One hour after adjusting the pH, the solution was checked and the pH re-adjusted if a change had taken place. The Base/Neutral and Acid fractions were then concentrated to one mL in a Kuderna-Danish apparatus and stored in the dark at 4°C until analysis.

The volatile organic compounds were isolated from the water samples using a purge and trap technique [20,21]. Five-mL aliquots were purged for 12 minutes at 40 mL per minute with high-purity helium and adsorbed on a TENAX-GC (Supelco, Bellefonte, PA) substrate. After this purge interval, a 180°C desorb cycle was activated for three minutes and the trapped compounds were backflushed onto the head of the GC column by a helium carrier gas flowing at a rate of 20 mL per minute. The temperature program and data acquisition were begun when the desorb cycle was initiated.

The priority pollutants in Aliquot Nos. 1 and 2 were identified using computer programs in the GC/MS data systems. These programs also measured the areas under extracted ion current profiles (EICP), i.e., mass chromatograms, at selected quantitation m/z 's (Table II) and normalized those areas to areas for internal standards. Anthracene- d_{10} was used as the internal standard for the Base/Neutral and Acid fractions. The Anthracene- d_{10} was added to the extract at a concentration of 20 $\mu\text{g/mL}$ immediately prior to injection into the GC/MS and was determined at m/z 188. Chlorobromomethane and 1,4-dichlorobutane, both at 20 $\mu\text{g/L}$, were used as internal standards for the volatile fractions. EICP areas for m/z 's 128 and 55, respectively, were used for normalization. The internal standard eluting with a retention time closest to the compound of interest was used in the quantitation process. Calibration curves consisting of relative response versus concentration were used to determine concentrations in the extracts.

These initial concentration values were subsequently corrected for the initial-to-final sample volume ratio so as to provide final results in $\mu\text{g/L}$. It should be noted that the calibration curves were prepared using the unlabeled spiking solutions as the reference in order to reduce systematic errors. Examples of the calibration curves are given in Figure 1a and 1b for toluene and phenol, respectively.

Both unlabeled and labeled priority pollutants in Aliquot Nos. 3 and 4 were identified automatically using computer programs in the GC/MS data systems. EICP areas were also automatically determined for the isotope ratio m/z 's specified in Table II. Using these areas, isotope ratios were calculated and concentrations determined using the direct proportionality method [6]. With this method, the concentration ratio of unlabeled-to-labeled compound is equal to the isotope ratio and calibration curves are not employed. The concentrations of labeled compounds in the spiking solutions were determined through comparisons with the unlabeled spiking solutions. This eliminates systematic errors which might be incorporated during the isotope ratio measurement process or which would result from low purities of the labeled compounds [9]. The m/z 's used for isotope ratio measurements were selected using the ion selection factor method [8].

RESULTS AND DISCUSSION

At the beginning of this study, it was recognized that not all priority pollutants had readily available stable isotopically labeled analogs. Consequently, a decision was made to limit the study to those compounds for which labeled analogs were available plus a limited number of additional compounds for which labeled analogs would be synthesized. The synthesized compounds were selected to be representative of compound classes not covered by the readily available labeled compounds. Because the synthesized compounds are now considered stock items by the manufacturers, they were not specifically identified in Table II. One compound class which was originally to be covered was the phthalate esters; unfortunately, synthesis was not completed in a time consistent with the study. Deuterated analogs were generally selected in favor of other labeled forms due to their lower cost. The deuterated chloroform and bromoform species, however, are known to exchange in water [22] to form the unlabeled compounds and consequently, ^{13}C analogs were selected. ^{13}C analogs were also employed when deuterated analogs were not feasible, i.e., carbon tetrachloride and pentachlorophenol.

Subsequent to acquiring the labeled compounds, it was necessary to select the most appropriate pair of isotope ratio m/z 's for each compound and its labeled analog. This was done according to the ion selection factor method [8]. Ion selection (ISF) factors were calculated (Table IV) for prominent m/z 's and the pair having the largest ISF value was selected for quantitation. There were some exceptions; for acenaphthene, fluorene, 2,4-dinitrophenol, pentachlorophenol, chlorobenzene, and toluene, molecular ions were chosen in preference to the m/z 's identified by the ISF criteria. In all these cases, the ISF values for the molecular ion pairs and the theoretically best m/z pair were similar and consequently, no significant impact on the results would be expected. The molecular ions were given preference to allow the measurements to take place at higher m/z 's where fewer interferences would be anticipated.

After acquiring spectral data, concentration values were calculated. The values obtained for Aliquot No. 1 were used to make background corrections to the values for Aliquot No. 2. Percent recovery values were then determined for the compounds spiked into Aliquot No. 2. Percent recoveries were also calculated for Aliquot No. 4 after background correction using the Aliquot No. 3 data. At this point, the recovery of each compound was checked for acceptability according to two criteria:

- (1) the absolute recovery had to be greater than two percent, and
- (2) the spiked concentration of a compound had to exceed the background concentration of that compound.

The first of these conditions was incorporated to eliminate data obtained below the quantitative detection limit for the methods and instruments employed. Because the detection limit is nominally based on the quantity of a compound which enters the mass spectrometer's ion source, absolute recoveries rather than relative recoveries were used for this test. This meant that raw EICP areas rather than nominalized areas had to be employed to determine recovery for the Base/Neutral and Acid fraction compounds in Aliquots No. 3 and 4. It should be noted that a two percent recovery corresponded to only 0.8 ng injected for Base/Neutral and Acid fraction compounds. For Volatile fraction compounds, absolute recoveries could not be determined owing to the inability to separate the sample preparation process, i.e., purge and trap, from the measurement process. Consequently, values were rejected because of low recovery only if a compound was undetectable in this fraction.

As can be seen in Table V, 28 values in the Base/Neutral fractions were excluded due to low recovery; of these, 12 were in Aliquot No. 2 and 16 in Aliquot No. 4. The interesting point, however, is that of the 28 low recovery values, only six were in corresponding sample aliquots while 22 were in apparently random aliquots. This tends to indicate a sample preparation effect rather than a matrix effect as the cause of these low recoveries. Furthermore, three compounds accounted

for 86 percent of the rejected values; apparently, the dichlorobenzenes and the nitrobenzene are not reproducibly recovered from the samples or they are being irreproducibly lost during sample preparation. In the Acid fractions, 105 low recovery values were encountered. Seventy-six percent of these were for 2,4-dinitrophenol, 2,4-dimethylphenol, and pentachlorophenol. 2,4-Dinitrophenol and pentachlorophenol are comparatively acidic compounds and seem to be lost on the gas chromatographic column. This was evidenced by much poorer detectability when standard solutions were injected on column. The 2,4-dimethylphenol, on the other hand, is comparatively nonacidic and tends to be extracted into the Base/Neutral fraction. For the rejected values associated with the remaining five phenols, 22 were common to Aliquot Nos. 2 and 4 and only 3 were not. This tends to indicate that low recovery for these compounds was primarily associated with sample matrix effects. In the Volatile fraction, only bromoform demonstrated a detection limit problem. This was apparently caused by decomposition in a heated stainless steel transfer line used by one of the laboratories.

Data were also rejected due to high background levels. This was done because of the propagation of error associated with the subtraction process [23]. Because the intent of the study was to evaluate the methods using real sample matrices, high background levels were of no interest and acted only as interferences. Unlike the data rejected due to low recovery, there was almost a perfect one-to-one correlation between data rejected in Aliquot No. 2 and its associated Aliquot No. 4 (Table V). In the ten cases where this was not true, the background levels were within about ten percent of the spiked levels. The majority of high background values were associated with methylene chloride. This was caused by the necessity of using some samples which had originally been intended only for Base/Neutral and Acid fraction preparations. The containers these samples were stored in had been rinsed with methylene chloride prior to use and apparently some remained in the containers even after the cleaning cycle.

Once the data had been evaluated for acceptability, mean percent recoveries and percent standard deviations were calculated for each compound (Table VI). In viewing this data, it should be recalled that compounds determined by isotope dilution and by Method 624 (Volatile fraction) are relative recoveries in that both priority pollutants and internal standards were subjected to complete analytical protocols. For the Base/Neutral and Acid fractions analyzed by Method 625, the internal standard was added just prior to the measurement step in the analysis and consequently, was not subjected to the sample preparation procedure. This means that the percent recoveries for Base/Neutral and Acid fraction compounds analyzed by Method 625 are absolute recoveries.

For all the relative recoveries, the mean calculated values are near 100 percent (Table VII). The isotope dilution values, however, are much closer to 100 percent than are the Method 624 Volatile fraction values. This was as expected. Matrix effects have been shown to produce different relative purging efficiencies for priority pollutants and internal standards [24], but no difference in relative purging efficiency was expected for a compound and its labeled analog. For the Base/Neutral and Acid compounds determined using Method 625, the effects of measuring absolute recovery are evident in that the mean recoveries are substantially less than 100 percent. These values, however, are typical of those obtained for a much larger selection of industrial effluent samples [25].

The differences in calculated percent recoveries for the different methods are significant in that they are a measure of analysis error. These differences, however, may not represent a problem in the intended application of the data if the ability to reproduce a given recovery is adequate for that application. A sample can always be analyzed as received plus spiked at some known level and the percent recovery evaluated. As long as recovery is reproducible, the calculated concentration determined for the unspiked aliquot can be adjusted to reflect percent recovery and an accurate value for the original

concentration can be produced. Although this approach is technically feasible, it requires (1) a doubling of the analysis effort and (2) having a knowledge of a compounds background concentration in order to assure spiking at an appropriate level. If a compound is spiked at a relatively high concentration, the percent recovery information may not be representative of recovery at a low background concentration and if the spiked concentration is low, the propagated error associated with subtraction becomes overwhelming. This means that the precision of the calculated percent recoveries given in Table VI is critical; the more precise method being of greater utility. As is readily apparent in Table VI, the percent standard deviations obtained using isotope dilution are much smaller than those obtained using Methods 624 and 625. For the Base/Neutral compounds evaluated, the mean precision (Table VII) was 4.7 times greater with isotope dilution than with Method 625. For the Acid compounds, the precision was 3.6 times greater, and for the Volatile fraction it was 4.3 times greater. It should be noted that the precision values obtained for Methods 624 and 625 are consistent with those which have been reported for a larger body of data [25]. This indicates that the isotope dilution method is relatively unaffected by sample matrix when compared with Methods 624 and 625.

In an attempt to evaluate whether Methods 624 and 625 would show improved precision with samples from a single industrial type, the results from the organic chemical industry effluents were evaluated independent of the other data. The results of this evaluation are shown in Table VIII. For the Base/Neutral compounds, isotope dilution is 5.6 times more precise and for the Acid compounds, it is 4.4 times more precise. These do not represent significant improvements in precision. The Method 624 data, however, do show a significant improvement; isotope dilution is now only 1.3 times more precise. It is not clear, however, whether this evaluation is significant; only five samples were involved and these originated from different sources.

An interesting and potentially useful aspect of analyses carried out using isotope dilution is the ability to perform an important quality assurance/quality control activity using the labeled analogs of the compounds of interest. The close relationship between the recoveries of a naturally abundant compound and its labeled analog suggests the use of the labeled species as a check on an absolute recovery. For example, if 100 μg of 4-chlorophenylphenylether is spiked into a 1L water sample but it is not detected in the sample extract, the method can be assumed inappropriate for the naturally abundant compound in that sample. In order to obtain the same recovery information without using the labeled compound, two sample extracts would have to be prepared as previously discussed. Also, the labeled analog can be used to define a very narrow retention time window within which the naturally abundant compound will elute. Methods 624 and 625 specify retention time windows of ± 60 seconds. This seems to present a much greater potential for false positive identifications to take place than the ± 3 second retention time window which has been found appropriate for the investigated priority pollutants based on the elution of their labeled analogs.

The most important observations of this study are that the precision and accuracy of calculated percent recoveries determined using isotope dilution are uniformly unaffected by sample matrix as long as sufficient material is recovered for measurement. This was not found to be the case with more conventional GC/MS methods, such as EPA Methods 624 and 625. Also highly significant was (1) the demonstration that large numbers of compounds can be identified and quantitated in environmental samples using isotope dilution methodology in a routine manner using full spectrum data and readily available instrumentation and (2) the identification of quality assurance/quality control functions which can be used with labeled analogs.

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Table I

GC/MS APPARATUS

GC/MS	duPont 321	Finnigan 1020	duPont 321	duPont 321	Finnigan 4000
Data Systems	Finnigan/INCOS	Finnigan 1020	Finnigan/INCOS	Finnigan/INCOS	Finnigan/INCOS
Location	S ³	S ³	TRW	TRW	TRW
Ionizing Voltage (V)	73	70	73	73	70
Emission Current (ua)	400	750	400	400	250
Mass Range A* (amu)	20-300	NA	NA	NA	22-450
Mass Range B** (amu)	47-500	47-500	47-500	47-500	NA
Scan Rate (sec/scan)	3	3	2	2	2

*For Volatile Organic Fraction

**For Base/Neutral and Acid Fractions

Table II
QUANTITATION DATA

Fraction	Method 624/5 m/z (19)	Concentration in Water Sample (ug/l)	Labeled Analog	Labeled Analog Source	Isotope Dilution m/z's	Labeled Analog Concentration in Water Sample (ug/l)
BASE/NEUTRAL						
acenaphthene	54	20	d ₁₀	MERCK	154/164	100
1,2-dichlorobenzene	146	20	d ₈	MERCK	146/152	100
1,4-dichlorobenzene	146	20	d ₈	MERCK	146/152	100
naphthalene	128	20	d ₈	MERCK	128/136	100
nitrobenzene	77	20	d ₅	MERCK	123/128	100
benzo(a)anthracene	220	20	d ₁₂	MERCK	228/240	100
benzo(a)pyrene	252	20	d ₁₂	MERCK	252/264	100
chrysene	228	20	d ₁₂	MERCK	228/240	100
anthracene	178	20	d ₁₀	MERCK	178/188	100
fluorene	166	20	d ₁₀	MERCK	166/176	100
pyrene	202	20	d ₁₀	MERCK	202/212	100
phenanthrene	178	20	d ₁₀	MERCK	178/188	100
4-chlorophenylphenylether	204	20	d ₅	MERCK	204/209	100
N-nitrosodiphenylamine	169	20	d ₆	MERCK	169/175	100
ALD						
2-nitrophenol	139	20	d ₈	KOR	139/143	100
2,4-dichlorophenol	162	24	d ₃	MERCK	162/167	120
2,4-dinitrophenol	184	20	d ₃	MERCK	184/187	100
2-chlorophenol	128	20	d ₈	MERCK	128/132	100
2,4-dimethylphenol	122	20	d ₃	MERCK	122/125	100
phenol	94	20	d ₅	MERCK	94/99	100
pentachlorophenol	266	20	¹³ C ₆	KOR	266/272	100
2,4,6-trichlorophenol	196	20	d ₂	MERCK	196/200	100
VOLATILE						
acrylonitrile	53	100	d ₃	MERCK	53/56	100
benzene	78	20	d ₆	MERCK	78/84	20
carbon tetrachloride	117	20	¹³ C	KOR	47/48	20
chlorobenzene	112	20	d ₅	MERCK	112/117	20
1,2-dichloroethane	98	20	d ₄	MERCK	62/65	20
1,1,1-trichloroethane	97	20	d ₃	MERCK	97/100	20
1,1,2-trichloroethane	97	20	d ₁	MERCK	83/84	20
1,1,2,2-tetrachloroethane	168	20	d ₂	MERCK	83/84	20
1,1-dichloroethylene	96	20	d ₂	MERCK	61/65	20
ethylbenzene	106	20	d ₁₀	MERCK	91/98	20
methylene chloride	84	20	d ₂	ALDRICH	84/88	20
bromoform	173	20	¹³ C	MERCK	173/174	20
toluene	92	20	d ₅	KOR	92/97	20
chloroform	83	20	¹³ C	MERCK	85/86	20

Table III.

SAMPLE SOURCES

<u>Industrial Type</u>	<u>Number of Samples</u>
Municipal Waste Water Treatment	2
Water Supply	1
Coal Mining	2
Gasohol Manufacturer	1
Leather Tanning	1
Molybdenum Mining	1
Uranium Mining	1
Pesticide Manufacturer	2
Blast Furnace	1
Steam Electric	1
Coal Gasification	1
Organic Chemical Manufacturer	5
Pulp and Paper Mill	1

Table IV. M/Z SELECTION

<u>Compound</u>	<u>Labeled Analog</u>	<u>Ratio m/z's</u>	<u>Ion Selection Factor</u>
<u>BASE/NEUTRALS</u>			
acenaphthene	acenaphthene, d ₁₀	153/162	9.04
		153/164	9.03
		154/162	8.82
		154/164	8.81
		152/162	8.76
		152/164	8.76
1,2-dichlorobenzene	1,2-dichlorobenzene, d ₄	146/152	8.30
		148/152	7.88
		111/115	7.50
		146/150	5.12
		148/150	4.74
		75/78	4.10
1,4-dichlorobenzene	1,4-dichlorobenzene, d ₄	146/152	8.17
		148/152	6.15
		146/150	5.05
		75/78	4.11
		148/150	3.05
naphthalene	naphthalene, d ₈	128/136	9.51
		129/136	8.39
		129/134	6.61
		51/54	4.97
nitrobenzene	nitrobenzene, d ₅	123/128	7.00
		77/82	6.66
		51/54	5.90
		50/54	4.87
benzo(a)anthracene and chrysene	benzo(a)anthracene and chrysene, d ₁₂	226/240	8.89
		226/238	8.25
		226/236	8.01
		228/240	7.34
		228/239	6.91
		228/238	6.72
fluorene	fluorene, d ₁₀	165/176	9.19
		165/174	9.19
		166/176	9.07
		166/174	9.07
		166/175	8.43

Table IV. (Continued)

<u>Compound</u>	<u>Labeled Analog</u>	<u>Ratio m/z's</u>	<u>Ion Selection Factor</u>
pyrene	pyrene, d ₁₀	202/212	9.79
		202/211	8.73
		200/210	7.58
		201/211	7.53
		203/213	7.29
anthracene and phenanthrene	anthracene and phenanthrene, d ₁₀	178/188	10.20
		177/188	9.40
		178/184	9.17
		177/187	8.15
		179/189	5.63
benzo(a)pyrene	benzo(a)pyrene, d ₁₂	252/264	9.98
		252/265	9.20
		253/265	8.21
4-chlorophenyl phenyl ether	4-chlorophenyl phenyl ether d ₅	204/209	7.45
		206/211	5.68
		141/146	3.65
N-nitrosodiphenylamine	N-nitrosodiphenylamine d ₆	168/175	7.39
		169/175	6.32
<u>LABELED ACID COMPOUNDS</u>			
2-chlorophenol	2-chlorophenol, d ₄	128/132	8.28
		130/132	7.56
		129/132	7.38
		63/68	4.01
		64/69	1.75
		64/66	1.60
2,4-dimethylphenol	2,4-dimethylphenol, d ₃	122/125	6.57
		122/124	6.01
		122/126	5.91
		107/110	5.47
		122/123	5.24
phenol	phenol, d ₅	94/99	9.30
		94/100	7.53
		66/71	5.97
		65/69	4.28
		65/70	2.77

Table IV. (Continued)

<u>Compound</u>	<u>Labeled Analog</u>	<u>Ratio m/z's</u>	<u>Ion Selection Factor</u>
2,4-dinitrophenol	2,4-dinitrophenol, d ₃	154/157	5.25
		184/187	5.15
		107/110	4.91
		63/66	1.76
2,4-dichlorophenol	2,4-dichlorophenol, d ₃	162/167	7.01
		162/165	6.56
		98/101	5.12
		164/167	2.37
2-nitrophenol	2-nitrophenol, d ₄	139/143	9.08
		81/85	8.34
		63/69	6.23
		65/69	5.34
2,4,6-trichlorophenol	2,4,6-trichlorophenol, d ₂	196/200	4.65
		196/198	4.14
		132/136	2.15
		97/99	0.780
pentachlorophenol	pentachlorophenol, ¹³ C ₆	165/170	5.01
		266/272	4.99
		266/270	4.02
		268/274	2.14
		268/272	1.18
<u>LABELED VOLATILE COMPOUNDS</u>			
acrylonitrile	acrylonitrile, d ₃	53/56	5.89
		51/54	4.56
benzene	benzene, d ₆	78/84	8.63
		51/56	7.33
		52/56	3.60
carbon tetrachloride	carbon tetrachloride, ¹³ C	47/48	6.24
		82/83	4.89
		82/85	4.03
		117/118	4.10
		119/120	2.57

Table IV. (Continued)

<u>Compound</u>	<u>Labeled Analog</u>	<u>Ratio m/z's</u>	<u>Ion Selection Factor</u>
chlorobenzene	chlorobenzene, d ₅	77/82	8.01
		112/117	7.34
		51/54	7.27
1,2-dichloroethane	1,2-dichloroethane, d ₄	62/65	7.72
		62/67	7.27
		64/67	6.35
		49/53	5.22
		49/51	3.39
1,1,1-trichloroethane	1,1,1-trichloroethane, d ₃	97/100	7.08
		99/102	6.30
		61/65	4.25
		61/63	1.86
1,1,2-trichloroethane	1,1,2-trichloroethane, d ₂	83/84	7.08
		97/98	6.63
		85/86	6.26
		99/100	5.80
1,1,2,2-tetrachloroethane	1,1,2,2-tetrachloroethane, d ₂	83/84	7.91
		85/86	7.16
1,1-dichloroethylene	1,1-dichloroethylene, d ₂	61/65	4.57
		96/98	3.43
		61/63	1.97
		98/100	1.21
ethylbenzene	ethylbenzene, d ₁₀	91/98	8.62
		106/116	5.91
		51/54	4.43
methylene chloride	methylene chloride, d ₂	84/88	5.30
		84/86	2.51
		86/88	2.42
		49/53	4.01
		49/51	1.28
bromoform	bromoform, ¹³ C	173/174	3.77
		175/176	3.11
		171/172	0.660

Table IV. (Continued)

<u>Compound</u>	<u>Labeled Analog</u>	<u>Ratio m/z's</u>	<u>Ion Selection Factor</u>
toluene	toluene, d ₅	91/96	8.25
		91/97	8.25
		91/95	8.12
		92/96	7.73
		92/97	7.73
		92/95	7.60
chloroform	chloroform, ¹³ C	85/86	4.90
		47/48	3.07
		83/84	2.60

Table V.
RESULTS OF DATA ACCEPTANCE TESTS

Compound	Rejected Data				Accepted Data	
	Below Detection Limit		High Background			
	624/5 ⁽¹⁾	ID ⁽²⁾	624/5	ID	624/5	ID
acenaphthene	-	-	-	-	20	20
1,2-dichlorobenzene	2	4	-	-	18	16
1,4-dichlorobenzene	2	4	-	-	18	16
naphthalene	-	1	-	-	20	19
nitrobenzene	5	7	-	-	15	13
benzo(a)anthracene and chrysene	-	-	-	-	20	20
benzo(a)pyrene	1	-	-	-	19	20
anthracene and phenanthrene	-	-	-	-	20	20
fluorene	-	-	-	-	20	20
pyrene	1	-	-	-	19	20
4-chlorophenyl phenyl ether	1	-	-	-	19	20
N-nitrosodiphenylamine	-	-	-	-	20	20
2-nitrophenol	4	5	-	-	16	15
2,4-dichlorophenol	-	-	1	2	19	18
2,4-dinitrophenol	14	14	-	-	6	6
2-chlorophenol	3	3	1	1	16	16
2,4-dimethylphenol	17	16	-	-	3	4
phenol	2	2	2	1	16	17
pentachlorophenol	6	13	-	-	14	7
2,4,6-trichlorophenol	3	3	-	-	17	17
acrylonitrile	-	-	-	-	20	20
benzene	-	-	1	1	19	19
carbon tetrachloride	-	-	-	-	20	20
chlorobenzene	-	-	-	-	20	20
1,2-dichloroethane	-	-	1	1	19	19
1,1,1-trichloroethane	-	-	-	-	20	20
1,1,2-trichloroethane	-	-	-	-	20	20
1,1,2,2-tetrachloroethane	-	-	-	-	20	20
1,1-dichloroethylene	-	-	-	-	20	20
ethylbenzene	-	-	-	-	20	20
methylene chloride	-	-	13	17	7	3
bromoform	10	10	-	-	10	10
toluene	-	-	3	3	17	17
chloroform	-	-	4	5	16	15

(1) EPA Methods 624 and 625 [19]

(2) Isotope dilution

Table VI.
RESULTS COMPARISON

Compound	Calculated Percent Recovery		Percent Standard Deviation	
	624/5 ⁽¹⁾	ID ⁽²⁾	624/5	ID
acenaphthene	55.2	98.9	51.5	8.2
1,2-dichlorobenzene	31.9	99.2	49.2	14.7
1,4-dichlorobenzene	34.7	102.9	35.8	14.2
naphthalene	30.4	103.8	43.0	10.9
nitrobenzene	128.7	99.2	132.2	25.2
benzo(a)anthracene and chrysene	50.8	98.3	48.7	16.9
benzo(a)pyrene	42.6	98.4	47.9	13.3
anthracene and phenanthrene	71.5	101.0	59.5	10.1
fluorene	68.1	97.5	56.4	7.3
pyrene	41.1	100.2	49.4	9.4
4-chlorophenyl phenyl ether	34.2	100.0	59.0	7.7
N-nitrosodiphenylamine	76.9	101.0	54.9	6.9
2-nitrophenol	70.4	108.3	55.2	13.5
2,4-dichlorophenol	48.9	102.9	68.0	16.7
2,4-dinitrophenol	45.7	102.3	94.4	30.5
2-chlorophenol	49.1	104.1	60.2	9.1
2,4-dimethylphenol	4.7	76.3	61.4	29.9
phenol	43.8	95.9	58.5	20.3
pentachlorophenol	95.4	99.1	92.2	21.6
2,4,6-trichlorophenol	54.8	103.8	50.0	10.0
acrylonitrile	87.2	100.4	22.0	4.8
benzene	106.3	99.9	25.8	6.6
carbon tetrachloride	151.4	97.6	38.9	11.9
chlorobenzene	104.4	102.8	25.8	3.8
1,2-dichloroethane	125.6	100.0	94.6	6.2
1,1,1-trichloroethane	108.6	101.4	19.3	8.7
1,1,2-trichloroethane	82.4	99.0	22.9	6.9
1,1,2,2-tetrachloroethane	79.4	99.4	46.8	6.7
1,1-dichloroethylene	97.5	95.6	31.8	6.9
ethylbenzene	68.7	101.0	18.2	14.9
methylene chloride	181.4	99.3	64.1	9.1
bromoform	318.9	99.1	100.2	7.4
toluene	99.7	90.7	17.4	15.6
chloroform	105.4	92.1	25.5	17.9

(1) EPA Methods 624 and 625 [19]

(2) Isotope Dilution

Table VII.

MEAN VALUE FOR TWENTY INDUSTRIES

Fraction	Mean Calculated Percent Recovery		Mean & SD	
	624/5 ⁽¹⁾	ID ⁽²⁾	624/5	ID
Base/Neutral	56.3	100.0	57.3	12.1
Acid	51.6	99.1	67.5	19.0
Volatile	122.6	98.5	39.5	9.2

(1) EPA Methods 624 and 625 [19]

(2) Isotope Dilution

Table VIII.

MEAN VALUES FOR FIVE CHEMICAL INDUSTRIES

Fraction	Mean Calculated Percent Recovery		Mean & SD	
	624/5 ⁽¹⁾	ID ⁽²⁾	624/5	ID
Base/Neutral	53.3	96.1	49.5	8.8
Acid	95.6	103.2	35.8	8.1
Volatile	102.0	96.6	15.5	11.5

(1) EPA Methods 624 and 625 [19]

(2) Isotope Dilution

FIGURES

Figure 1 Relative response calibration curve for (a) Phenol and (b) Toluene.
The dotted lines enclose a ± 10 percent error window.

PHENOL CALIBRATION CURVE

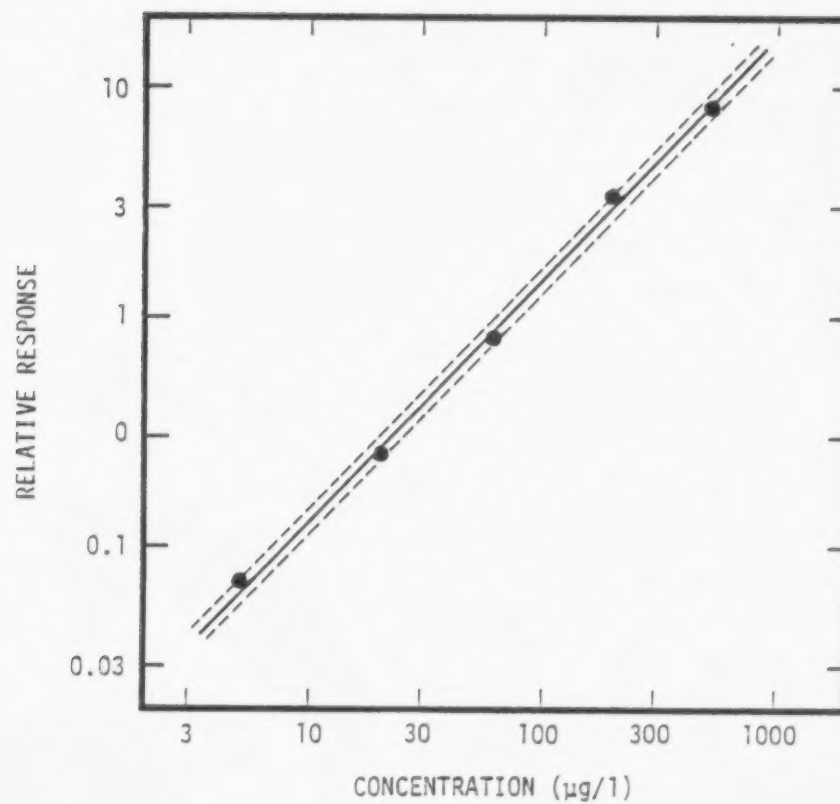


Figure 1a

TOLUENE CALIBRATION CURVE

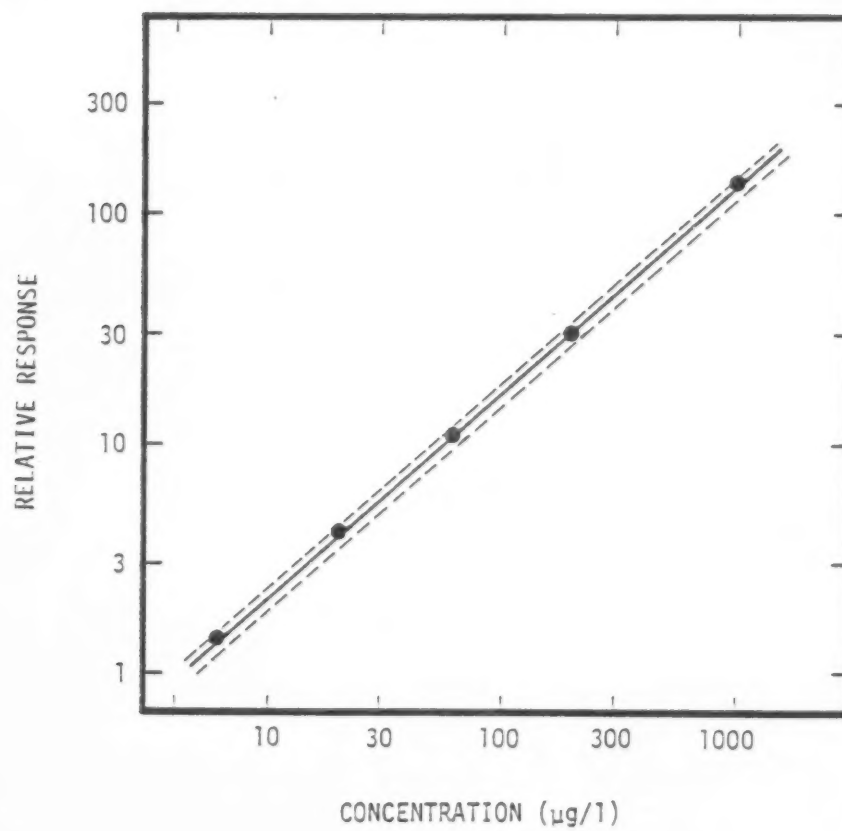


Figure 1b

APPENDIX B

INTERIM DRAFT - METHOD 1624 VOLATILE ORGANIC COMPOUNDS BY PURGE AND TRAP ISOTOPE DILUTION GC-MS

1. SCOPE AND APPLICATION

- 1.1 This method is designed to determine those "unambiguous priority pollutants," associated with the Consent Decree, that are amenable to the purge and trap method. The chemical compounds listed in Table 1 may be determined by this method.
- 1.2 This method is applicable to the determination of these compounds in municipal and industrial discharges. It is designed to be used to meet the survey requirements of the Effluent Guidelines Division (EGD) and the requirements of the National Pollutants Discharge Elimination System (NPDES).
- 1.3 The detection limit of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limits listed in Table 2 represent sensitivities that can be achieved in wastewaters.
- 1.4 The GC-MS parts of this method are recommended for use only by persons experienced in GC-MS analysis or under the close supervision of such qualified persons.

2. SUMMARY OF METHOD

2.1 A sample of wastewater is purged with a stream of inert gas. The gas is bubbled through a 5-ml water sample contained in a specially designed purging chamber. The volatile organic compounds are efficiently transferred from the aqueous phase into the gaseous phase where they are passed through a sorbent bed designed to trap out the volatile organic compounds. After purging is complete, the trap is backflushed while being heated rapidly to desorb the components thermally into the inlet of a gas chromatograph. The components are separated via the gas chromatograph and detected using a mass spectrometer which is used to provide both qualitative and quantitative information. The chromatographic conditions as well as typical mass spectrometer operating parameters are supplied.

3. INTERFERENCES

3.1 Interferences coextracted from the samples will vary considerably from source to source, depending upon the diversity of the industrial complex or municipality being sampled. Impurities in the purge gas and organic compounds out-gassing from the plumbing ahead of the trap account for the majority of contamination problems. The analytical system must be demonstrated to be free from the interferences under the conditions of the analysis by running method blanks. Method blanks are run by charging the purging device with organic compound-free water and analyzing it in the normal manner. The use of non-TFE plastic tubing, non-TFE thread sealants, or flow controllers with rubber components in the purging device should be avoided.

3.2 Samples can be contaminated by diffusion of volatile organic materials (particularly methylene chloride) through the septum seal into the sample during shipment and storage. A field blank prepared from organic compound-free water and carried through the sampling and handling protocol can serve as a check on such contamination.

3.3 Cross-contamination can occur whenever high-level and low-level samples are analyzed sequentially. To reduce cross-contamination, it is recommended that the purging device and sample syringe be rinsed twice, between samples, with organic compound-free water to check for cross-contamination. For samples containing large amounts of water soluble materials, suspended solids, high-boiling compounds or high-organohalide levels, it may be necessary to wash the purging device with a soap solution, rinse with distilled water, and then dry in a 105°C oven between analyses.

4. APPARATUS AND MATERIALS

4.1 Sampling equipment for discrete sampling.

4.1.1 Vial, with cap - 40-ml capacity screw cap (Pierce No. 13075 or equivalent). Wash the vial with detergents, rinse it with water and then dry it at 105°C for one hour before use.

4.1.2 Septum - Teflon-faced silicone (Pierce No. 12722 or equivalent). Wash the septum with detergents, rinse it with water and then dry it at 105°C for one hour before use.

4.2 Purge and trap device - The purge and trap equipment consists of three separate pieces of apparatus: the purging device, trap, and desorber. Several complete devices are now available commercially. The device must meet the following specifications: The unit must be completely compatible with the gas chromatographic system; the purging chamber must be designed for a 5-ml volume and be modeled after Figure 1; the dimensions for the sorbant portion of the trap must meet or exceed those in Figure 2. Figures 3 and 4 illustrate the complete system in the purge and the desorb mode.

4.3 Gas chromatograph - Analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including an analytical column.

4.3.1 Column 1 - An 8-ft glass column (1/4 in OD x 2 mm ID) packed with 1 percent SP-1000 coated on 60/80 mesh Carbopak B preceded by a 6-cm precolumn packed with 1 percent SP-1000 coated on 60/80 mesh Chromosorb W. The precolumn is necessary only during the conditioning step.

4.3.2 Column 2 - An 8-ft glass column (1/4 in OD x 2 mm ID) packed with 0.2 percent Carbowax 1500 coated on 60/80 mesh Carbopak C preceded by a 1-ft precolumn packed with 3 percent Carbowax 1500 coated on 60/80 mesh Chromosorb W. The precolumn is necessary only during the conditioning step.

4.4 Syringes - glass 5-ml hypodermic syringe with Luer-Lok tip (3 each)

- 4.5 Micro syringes - 10 μ l, 25 μ l, and 100 μ l sizes.
- 4.6 Two-way syringe valve with Luer Lok ends (3 each, Teflon or Kel-F).
- 4.7 Syringe - 5-ml, gas-tight with shut-off valve.
- 4.8 Eight-inch, 20-gauge syringe needle - one per each 5-ml syringe.
- 4.9 Mass Spectrometer - capable of scanning from 20-260 a.m.u. in three seconds or less with an electron energy of 70 volts (nominal), and producing a recognizable mass spectrum (background corrected) at unit resolution from 20 ng of 4-bromofluorobenzene (BFB) when injected through the GC inlet. The mass spectrometer must be interfaced with a gas chromatograph equipped with an inlet system designed for purge and trap work. All sections of the transfer lines must be glass or glass-lined and deactivated; use Sylon-CT, Supelco (or equivalent), to deactivate. The GC-MS interface can utilize any separator that gives recognizable mass spectra (background corrected) and acceptable calibration points at the limit of detection specified for each compound in Table 2.
- 4.10 A computer system should be interfaced to the mass spectrometer to allow acquisition of continuous mass scans for the duration of the chromatographic program. The computer system should also be equipped with mass storage devices for saving all data from GC-MS runs. There should be computer software available to allow searching any GC-MS run for specific ions and plotting the intensity of the ions with respect to time or scan number. The ability to integrate the area under a specific ion plot peak is essential for quantification.

5. REAGENTS

5.1 Sodium thiosulfate - (ACS) Granular.

5.2 Trap Materials

5.2.1 Porous polymer packing 60/80 mesh chromatographic grade Tenax GC (2,6-diphenylene oxide).

5.2.2 Three percent OV-1 on Chromosorb-W 60/80 mesh.

5.2.3 Silica gel - (35-60 mesh) - Davison, grade-15 or equivalent.

5.3 Activated carbon - Filtrasorb-200 (Calgon Corp.) or equivalent.

5.4 Organic compound-free water

5.4.1 Organic compound-free water is defined as water free of interference when employed in the purge and trap procedure described here. It can be generated by passing tap water or well water through a carbon filter bed containing about one pound of activated carbon.

5.4.2 Alternatively, water system (Millipore Super-Q or equivalent) may be used to generate organic compound-free water.

5.4.3 Organic compound-free water may also be prepared by boiling water for 15 minutes. Subsequently, while maintaining the temperature at 90°C, sparge the water for one hour using a contaminant-free inert gas. While still hot, transfer the water to a narrow mouth screw cap bottle equipped with a Teflon seal.

5.5 Stock standards (2 mg/ml) - Prepare stock standard solutions in methanol using assayed liquids or gases as appropriate. Because of the toxicity of some of the organohalides, primary dilutions of these materials should be prepared in a hood. A NIOSH/MESA-approved toxic gas respirator should be worn when the analyst handles high concentrations of such materials.

5.5.1 Place about 9.8 ml of methanol into a 10-ml ground glass stoppered volumetric flask. Allow the flask to stand, unstoppered, for about ten minutes or until all alcohol-wetted surfaces have dried. Tare the flask to the nearest 0.1 mg.

5.5.2 Add the assayed reference material:

5.5.2.1 Liquids - Using a 100- μ l syringe, immediately add two to three drops of assayed reference material to the flask, then reweigh the flask and contents. Be sure that the drops fall directly into the alcohol without contacting the neck of the flask.

5.5.2.2 Gases - To prepare standards of bromomethane, chloroethane, chloromethane, and vinyl chloride, fill a 5-ml valved gas-tight syringe with the reference standard to the 5.0-ml mark. Lower the needle to 5 mm above the methyl alcohol meniscus. Slowly inject the reference standard into the neck of the flask (the heavy gas will rapidly dissolve into methyl alcohol).

- 5.5.3 Reweigh the flask, dilute to volume, stopper, then mix by inverting the flask several times. Transfer the standard solution to a 15-ml screw-cap bottle equipped with a Teflon cap liner.
- 5.5.4 Calculate the concentration in mg per ml (equivalent to μg per μl) from the net gain in weight.
- 5.5.5 Store stock standards at 4°C. Prepare fresh standards every second day for the four gases and 2-chloroethylvinyl ether. All other standards must be replaced with a fresh standards each week. Remove samples from cold storage (approximately an hour prior to analysis) and bring to temperature in a water bath at 20 to 25°C.
- 5.6 Labeled compound spiking solution - From stock standard solutions prepared as above, prepare the spiking solution such that it contains 20 $\mu\text{g}/\text{ml}$ of each labeled compound in methanol. Acrylonitrile and acrolein should be at 100 $\mu\text{g}/\text{l}$. Spike 5 μl of this solution into each 5-ml sample and calibration standard analyzed.

6. CALIBRATION

- 6.1 Using stock standards, prepare secondary dilution standards in methanol that contain the compounds of interest, either singly or mixed together. The standards should be at concentrations such that aqueous standards prepared in 6.2 will completely bracket the working range of the chromatographic system. If the limit of detection listed in Table 2 is 10 $\mu\text{g}/\text{l}$, for example, prepare secondary methanolic standards at 100 $\mu\text{g}/\text{l}$, and 500 $\mu\text{g}/\text{l}$, so that aqueous standards prepared from these secondary calibration standards, and the primary standards, will define the linearity of the detector in the working range.

- 6.2 Using both the primary and secondary dilution standards, prepare calibration standards by carefully adding 20.0 μ l of the standard in methanol to 100, 500, or 1000 ml of organic compound-free water. A 25- μ l syringe (Hamilton 702N or equivalent) should be used for this operation. These aqueous standards must be prepared fresh daily.
- 6.3 Assemble the necessary gas chromatographic apparatus and establish operating parameters equivalent to those indicated in Table 2. By injecting secondary dilution standards, establish the linear range of the analytical system for each compound and demonstrate that the analytical system meets the limits of detection requirements in Table 2.
- 6.4 Assemble the necessary purge and trap device. Pack the trap as shown in Figure 2 and condition overnight at a nominal 180°C by backflushing with an inert gas flow 20 ml/min. Prior to use, condition the traps daily for 10 minutes by backflushing at 180°C. Analyze aqueous calibration standards (6.2) according to the purge and trap procedure in Section 9. Compare the responses to those obtained by injection of standards (6.3), to determine the analytical precision. The analytical precision of the analysis of aqueous standards must be comparable to data presented by Bellar and Lichtenberg (1978, Reference 3) before reliable sample analysis may begin.
- 6.5 The isotope dilution approach is used for the purgeable organic compounds when appropriate labeled compounds are available and when interferences do not preclude the analysis. If labeled compounds are not available, the analysis should proceed according to Method 624. A calibration curve encompassing the concentration range of interest is prepared for each compound to be determined.

When compounds having more than one order of magnitude in concentration are analyzed, plot relative response (RR) versus concentration ($\mu\text{g/l}$) on log-log paper or use a logarithmically weighted linear regression to fit the data. If a smaller concentration range is to be covered, linear plots and simple linear regressions are acceptable. An example of a calibration curve for toluene using toluene- d_5 is given in Figure 5. Also shown in Figure 5 are the ± 10 percent error limits (dotted lines). Relative Response is determined according to the procedures described below. A minimum of three data points are required for calibration but more points are desirable.

6.5.1 The relative response (RR) of naturally abundant compound-to-enriched compound is determined from isotope ratio values calculated from acquired data. Three isotope ratios are used in this process:

R_x = the isotope ratio of pure naturally abundant compound,

R_y = the isotope ratio of pure enriched compound and,

R_m = the isotope ratio of the analytical mixture of naturally abundant and enriched compounds.

The correct way to calculate RR is:

$$RR = \frac{(R_y - R_m)(R_x + 1)}{(R_m - R_x)(R_y + 1)}$$

If R_m is not between $2R_y$ and $0.5R_x$, the sample should be diluted and reanalyzed.

6.5.2 In most cases, the retention times of enriched and naturally abundant compounds are the same and isotope ratios (R's) can be calculated from the extracted ion current profiles where:

$$R = \frac{\text{Area at } m/z_1}{\text{Area at } m/z_2}$$

If either of the areas is zero, it is assigned a value of one in the calculations; that is if:

$$\begin{aligned} \text{area of } m/z_1 &= 50721 \text{ and} \\ \text{area of } m/z_2 &= 0 \end{aligned}$$

$$\text{then } R = \frac{50721}{1} = 50720$$

The m/z 's are always selected such that $R_x > R_y$.

When there is a difference in retention times (RT) between the naturally abundant and enriched compounds, some special precautions are required in order to determine the isotope ratios. First, R_x , R_y and R_m are defined as:

$$R_x = \frac{\text{Area } m/z_1 \text{ (at } RT_1 \text{)}}{1}$$

$$R_y = \frac{1}{\text{Area } m/z_2 \text{ (at } RT_2 \text{)}}$$

$$R_m = \frac{\text{Area } m/z_1 \text{ (at } RT_1 \text{)}}{\text{Area } m/z_2 \text{ (at } RT_2 \text{)}}$$

6.5.3 An example of the above calculations can be taken from the data plotted in Figure 6 for toluene and toluene-d₅. For these data,

$$R_x = \frac{168290}{1} = 168300$$

$$R_y = \frac{1}{60960} = .00001640$$

and

$$R_m = \frac{96868}{82508} = 1.174$$

The RR for the above data is then calculated using the equation given in 6.5.1. For example, RR = 1.174. It should be noted that not all isotopically enriched compounds elute before their naturally abundant analogs.

7. QUALITY CONTROL

7.1 Before processing any samples, demonstrate through the analysis of a method blank that all glassware and reagents are interference-free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against chronic laboratory contamination.

7.2 Standard quality assurance practices should be used with this method. Field replicates should be collected to determine the precision of the sampling technique. Laboratory replicates should be analyzed to determine the precision of the analysis. Fortified samples should be analyzed to determine the accuracy of the analysis.

7.3 The analyst should maintain constant surveillance of both the performance of the analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each 5-ml sample and blank with the enriched compounds. The isotopically enriched compounds should be used as monitors of performance; if recoveries change significantly, the cause should be determined and the system altered, if necessary.

8. SAMPLING COLLECTION, PRESERVATION, AND HANDLING

8.1 Grab samples must be collected in glass containers having a total volume greater than 20 ml. Fill the sample bottles in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottles so that no air bubbles are entrapped in it. Maintain the hermetic seal on the sample bottle until time of analysis.

8.2 The sample must be iced or refrigerated from the time of collection until extraction. If the sample contains residual chlorine, add sodium thiosulfate preservative (10 mg/40 ml) to the empty sample bottles just prior to shipping to the sample site, fill with sample just to overflowing, seal the bottle, and shake vigorously for one minute.

8.3 All samples must be analyzed within 7 days of collection.

9. SAMPLE EXTRACTION AND GAS CHROMATOGRAPHY

- 9.1 Remove standards and samples from cold storage (approximately an hour prior to an analysis) and bring to temperature in a water bath at 20-25°C.
- 9.2 Adjust the purge gas (nitrogen or helium) flow rate to 40 ml/min. Attach the trap inlet to the purging device, and set the device to the purge mode (Figure 3). Open the syringe valve located on the purging device sample introduction needle (Figure 1).
- 9.3 Remove the plunger from a 5-ml syringe and attach a closed syringe valve. Open the sample bottle (or standard) and carefully pour the sample into the syringe barrel until it overflows. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 ml. Because this process of taking an aliquot destroys the validity of the sample for future analysis, the analyst should fill a second syringe at this time to protect against possible loss of data. Add 5.0 μ l of the labeled compound spiking solution (5.6) through the valve bore, then close the valve.
- 9.4 Attach the syringe-valve assembly to the syringe valve on the purging device. Open the syringe valve and inject the sample into the purging chamber.
- 9.5 Close both valves and purge the sample for 12.0 ± 0.05 minutes.
- 9.6 After the 12-minute purge time, attach the trap to the chromatograph and adjust the device to the desorb mode. Introduce the trapped materials to the GC column by rapidly heating the trap to 180°C while backflushing the trap, with

an inert gas, at 20 to 60 ml/min for four minutes. If rapid heating cannot be achieved, the gas chromatographic column must be used as a secondary trap by cooling it to 30°C (or subambient, if problems persist) instead of the initial program temperature of 45°C.

- 9.7 While the volatile contents of the trap are being desorbed into the gas chromatograph, empty the purging chamber using the sample introduction syringe. Wash the chamber with two 5-ml flushes of organic compound-free water. After the purging device has been emptied, continue to allow the purge gas to vent through the chamber until the frit is dry, and ready for the next sample.
- 9.8 After desorbing the sample for four minutes, recondition the trap by returning the purge and trap device to the purge mode. Wait 15 seconds then close the syringe valve on the purging device to begin gas flow through the trap. Maintain the trap temperature at 180°C. After approximately seven minutes, turn off the trap heater and open the syringe valve to stop the gas flow through the trap. When cool, the trap is ready for the next sample. (Note: If this bake-out step is omitted, the amount of water entering the GC-MS system will progressively increase and may cause deterioration and potential shutdown of the system.)
- 9.9 The analysis of blanks is most important in the purge and trap technique because the purging device and the trap can be contaminated by residues from very concentrated samples or by vapors in the laboratory. Prepare blanks by filling a sample bottle with organic compound-free water that has been prepared by passing distilled water through a pretested activated carbon column. Blanks should be sealed, stored at 4°C, and analyzed with each group of samples.

10. GAS CHROMATOGRAPHY - MASS SPECTROMETRY

10.1 Table 2 summarizes the recommended gas chromatographic column materials and operating conditions for the instrument. Included in this table are estimated retention times and sensitivities. An example of the separation achieved by Column 1 is shown in Figure 7.

10.2 GC-MS Determination - Analytical conditions for determination of the priority pollutants amenable to purge and trap, using the Tekmar LCS-1 and a GC-MS are given below. Operating conditions may vary from one system to another.

10.2.1 Purge Parameters

Sample size - 5.0 ml.

Purge gas - Helium, high-purity grade.

Purge time - 12 minutes.

Purge flow - 40 ml/min.

Trap Dimensions - 1/8 in. OD (0.105 in. ID) x 25 cm long.

Trap sorbent - Tenax-GC, 60/80 mesh (7.7 cm), Type 15 silica gel, 35/60 mesh (7.7 cm) plus activated charcoal (7.7 cm).

Desorption flow - 20 ml/min.

Desorption time - 4 minutes.

Desorption temperature - 180°C.

10.2.7 Mass Spectrometer Parameters

Electron energy - 70 volts (nominal).

Mass range - 20-27, 33-260 amu.

Scan time - 3 seconds or less.

10.3 Calibration of the gas chromatography-mass spectrometry (GC-MS) system - Evaluate the system performance each day that it is used for the analysis of samples or blanks by injecting 20 ng of BFB into the GC inlet. Check to be sure that the performance criteria listed in Table 5 are met. If the criteria are not met, the instrument must be retuned to satisfy those criteria before continuing.

10.4 Analyze a 20 µg/l standard to demonstrate instrument performance (5.6).

11. QUALITATIVE AND QUANTITATIVE DETERMINATION

11.1 Identification of naturally abundant compounds is accomplished by (1) comparison of extracted ion current profile (EICP) areas with those of known standards and (2) evaluation of the retention time difference between the naturally abundant compound and its labeled analog.

11.1.1 EICP areas for the characteristic masses given in Table 4 must agree within twenty percent of those of a known standard. If a spectral interference is suspected, additional masses should be investigated.

11.1.2 The retention time difference between the naturally abundant compound and its labeled analog must agree within $\pm$ six seconds of those of known standards.

11.2 Identification of labeled compounds is accomplished by (1) comparison of EICP areas with those of known standards, and (2) evaluation of retention time.

11.2.1 EICP areas for the characteristic masses given in Table 4 must agree within twenty percent with those of a known standard. If a spectral interference is suspected, additional masses should be investigated.

11.2.2 The retention time of the labeled compound must agree within ± 60 seconds with those of a known standard.

11.3 Quantitative Calculations - By adding a known amount of each isotopically enriched compound to be analyzed to every sample prior to extraction, automatic correction for component recovery can be made. This is because the enriched and naturally abundant compound will exhibit the same matrix effects upon purging and desorption, etc. Relative response (RR) values for sample mixtures are used in conjunction with the calibration curves described in Section 6.5 to determine concentrations directly as long as the isotopically enriched compound spiking levels are constant. For the toluene (6.5.3) example given in Figure 6, RR would equal to 1.174. For this RR value, the toluene calibration curve given in Figure 5 indicates a concentration of 31.8 $\mu\text{g/l}$.

11.3.1 For isotope dilution analyses, the primary pairs of m/z 's for quantitation are given in Table 5. If there are interferences at these m/z 's or if an alternative labeled compound is employed, the best pair of m/z 's can be predicted by calculating an ion selection factor (ISF) where:

$$\text{ISF} = \log \frac{R_x}{R_y} - \log \frac{200}{a_x + b_y}$$

R_x and R_y are defined in Section 6.5.1 and a_x and b_y are the percent of total ionization values for the more abundant m/z 's of the natural and enriched compounds, respectively. Generally, the best m/z 's are found among the more abundant m/z 's in the spectra. Some ISF's for 1,2-dichloroethane and 1,2-dichloroethane- d_4 are given below.

m/z 's	R_x	R_y	a_x	b_y	ISF
62/67	553000	.000264	37.8	11.9	8.72
64/67	182000	.000164	12.0	11.9	8.12
98/104	74900	.00209	5.21	3.25	6.18
49/53	112000	.0206	15.3	4.87	5.74
98/102	12.6	.00126	5.21	5.22	2.72

For these two compounds, isotope ratios made at m/z 's of 62 and 67 should be the best.

11.4 Record all results to three significant figures. Record labeled compound data to two significant figures. When duplicate and spiked samples are analyzed, all data obtained should be reported. Report results in micrograms per liter. Include identification of any components found in the blanks.

12. ACCURACY AND PRECISION

12.1 Precision of the method is determined by preparing and analyzing replicate sample aliquots. Accuracy of the method is determined by analyzing samples spiked with the compounds of interest.

12.2 See Reference 9.

13. REFERENCES

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Table 1

VOLATILE ORGANIC COMPOUNDS ANALYZED BY
PURGE AND TRAP ISOTOPE DILUTION (GC/MS) METHOD

<u>Compound</u>	<u>STORET No.</u>
Acrolein	34210
Acrylonitrile	34215
Benzene	34236
Bromomethane	34413
Bromodichloromethane	32101
Bromoform	32104
Carbon tetrachloride	32102
Chlorobenzene	34301
Chloroethane	34311
2-Chloroethylvinyl ether	34576
Chloroform	32106
Chloromethane	34418
Dibromochloromethane	34105
1,1-Dichloroethane	34496
1,2-Dichloroethane	34531
1,1-Dichloroethene	34501
trans-1,2-Dichloroethene	34546
1,2-Dichloropropane	34541
cis-1,3-Dichloropropene	34561
trans-1,3-Dichloropropene	34561
Ethylbenzene	34371
Methylene chloride	34423
1,1,2,2-Tetrachloroethane	34516
Tetrachloroethene	34475
1,1,1-Trichloroethane	34506
1,1,2-Trichloroethane	34511
Trichloroethene	39180
Trichlorofluoromethane	34488
Toluene	34010
Vinyl chloride	39175

Table 2

GAS CHROMATOGRAPHY OF ORGANIC COMPOUNDS BY
PURGE AND TRAP ISOTOPE DILUTION (GC/MS) METHOD

Compound	Retention Time (min)		Limit of Detection* ($\mu\text{g/l}$)
	Column 1	Column 2	
chloromethane	1.50	2.10	10
bromomethane	2.17	2.50	10
vinyl chloride	2.67	2.57	10
chloroethane	3.33	2.82	10
methylene chloride	5.25	4.03	10
trichlorofluoromethane	7.18	5.14	10
1,1-dichloroethene	7.92	5.25	10
1,1-dichloroethane	9.30	6.48	10
trans-1,2-dichloroethene	10.08	6.81	10
chloroform	10.68	7.70	10
1,2-dichloroethane	11.40	8.29	10
1,1,1-trichloroethane	12.60	9.28	10
carbon tetrachloride	13.03	9.45	10
bromodichloromethane	13.65	10.36	10
1,2-dichloropropane	14.92	11.30	10
trans-1,3-dichloropropene	15.22	11.70	10
trichloroethene	15.80	11.98	10
dibromochloromethane	16.48	12.86	10
1,1,2-trichloroethane	16.52	12.86	10
cis-1,3-dichloropropene	16.53	12.86	10
benzene	-	12.95	10
2-chloroethylvinyl ether	18.00	13.71	10
bromoform	19.23	15.41	10
1,1,2,2-tetrachloroethane	21.62	17.70	10
tetrachloroethene	21.67	17.44	10
toluene	-	18.53	10
chlorobenzene	24.18	20.57	10
ethylbenzene		25.06	10
acrolein			100
acrylonitrile			100

Column 1: Eight ft. glass column (1/4 in OD x 2 mm ID) packed with one percent SP-1000 coated on 60/80 Carbopak B. Carrier gas helium at 40 ml/min. Temperature program: 3 min. isothermal at 45°C, then 8°/min to 220°, hold at 220° for 15 minutes.

Column 2: Eight ft. glass column (1/4 in. OD x 2 mm ID) packed with 0.2 percent Carbowax 1500 coated on 60/80 mesh Carbopak C. Carrier gas: helium at 40 ml/min. Temperature program: 3 min. isothermal at 60°C then 8°/min to 160°, hold at 160 until all compounds elute.

*This is a minimum level at which the entire system must give recognizable mass spectra (background corrected) and acceptable calibration points.

Table 3
BFB KEY IONS AND ION ABUNDANCE CRITERIA

<u>Mass</u>	<u>Ion Abundance Criteria</u>
50	20 to 40 pct of mass 95.
75	50 to 70 pct of mass 95.
95	Base peak, 100 pct relative abundance.
96	5 to 9 pct of mass 95
173	Less than 1 pct of mass 95.
174	70 to 90 pct of mass 95.
175	5 to 9 pct of mass 95.
176	70 to 90 pct of mass 95.
177	5 to 9 pct of mass 95.

Table 4
CHARACTERISTIC IONS OF THE VOLATILE ORGANIC COMPOUNDS

Compound	Characteristic Masses				Labeled Analog	Characteristic Masses			
chloromethane	50	52			*	*			
bromomethane	94	96			*	*			
vinyl chloride	62	64			*	*			
chloroethane	64	66			*	*			
methylene chloride	49	51	84	86	d ₂	53	54	90	
trichlorofluoromethane	101	103			*	*			
1,1-dichloroethene	61	96	98		d ₂	65	100	102	
1,1-dichloroethane	63	65	83		*	*			
	85	98	100		*	*			
trans-1,2-dichloroethene	61	96	98		*	*			
chloroform	83	85			¹³ C	84	86		
1,2-dichloroethane	62	64	98	100	d ₄	65	67	53	
1,1,1-trichloroethane	97	99	117	119	d ₃	100	102	63	
carbon tetrachloride	117	119	121		¹³ C	118	120	83	
bromodichloromethane	83	85	127	129	*	*			
1,2-dichloropropene	63	65	112	114	*	*			
trans-1,3-dichloropropene	75	77			*	*			
trichloroethene	95	97	130	132	*	*			
dibromochloromethane	129	127	208	206	*	*			
cis-1,3-dichloropropene	75	77			*	*			
1,1,2-trichloroethane	83	85	97		d ₁	84	86	98	100
	99	132	134						
benzene	78	77	51	52	d ₆	84	82	54	56
2-chloroethylvinyl ether	63	65	106		*	*			
bromoform	171	173	175		¹³ C	172	174	176	
	252	254				253	255		
tetrachloroethene	129	131	164	166	*	*			
1,1,2,2-tetrachloroethane	83	85	131	133	d ₂	84	86	88	
	166	168							
toluene	91	92	51		d ₅	95	97	54	
chlorobenzene	112	114	77		d ₅	117	119	82	
ethylbenzene	91	106	51		d ₁₀	98	116	54	
acrolein	26	27	55	56	*	*			
acrylonitrile	26	51	52	53	d ₃	54	56	57	

*Data not available at this time.

Table 5
ISOTOPE DILUTION IONS FOR VOLATILE ORGANIC COMPOUNDS

<u>Compound</u>	<u>Isotope Labeled Analog</u>	<u>Ratio m/z's</u>
chloromethane	*	*
bromomethane	*	*
vinyl chloride	*	*
chloroethane	*	*
methylene chloride	methylene chloride, d ₂	84/88
trichlorofluoromethane	*	*
1,1-dichloroethane	*	*
trans-1,2-dichloroethane	*	*
chloroform	chloroform, ¹³ C	85/86
1,2-dichloroethane	1,2-dichloroethane-d ₄	62/67
1,1,1-trichloroethane	1,1,1-trichloroethane, d ₃	97/100
carbon tetrachloride	carbon tetrachloride, ¹³ C	47/48
bromodichloromethane	*	*
1,2-dichloropropane	*	*
trans-1,3-dichloropropene	*	*
trichloroethene	*	*
dibromochloromethane	*	*
cis-1,3-dichloropropene	*	*
1,1,2-trichloroethane	1,1,2-trichloroethane, d ₁	83/84
benzene	benzene-d ₆	78/84
2-chloroethylvinyl ether	*	*
bromoform	bromoform, ¹³ C	173/174
tetrachloroethene	*	*
1,1,2,2-tetrachloroethane	1,1,2,2-tetrachloroethane, d ₂	83/84
toluene	toluene-d ₅	92/97
chlorobenzene	chlorobenzene, d ₅	112/117
ethylbenzene	ethylbenzene-d ₁₀	91/98
acrolein	*	*
acrylonitrile	acrylonitrile, d ₃	53/56
1,1-dichloroethylene	1,1-dichloroethylene, d ₂	61/65

*Data unavailable at time of writing.

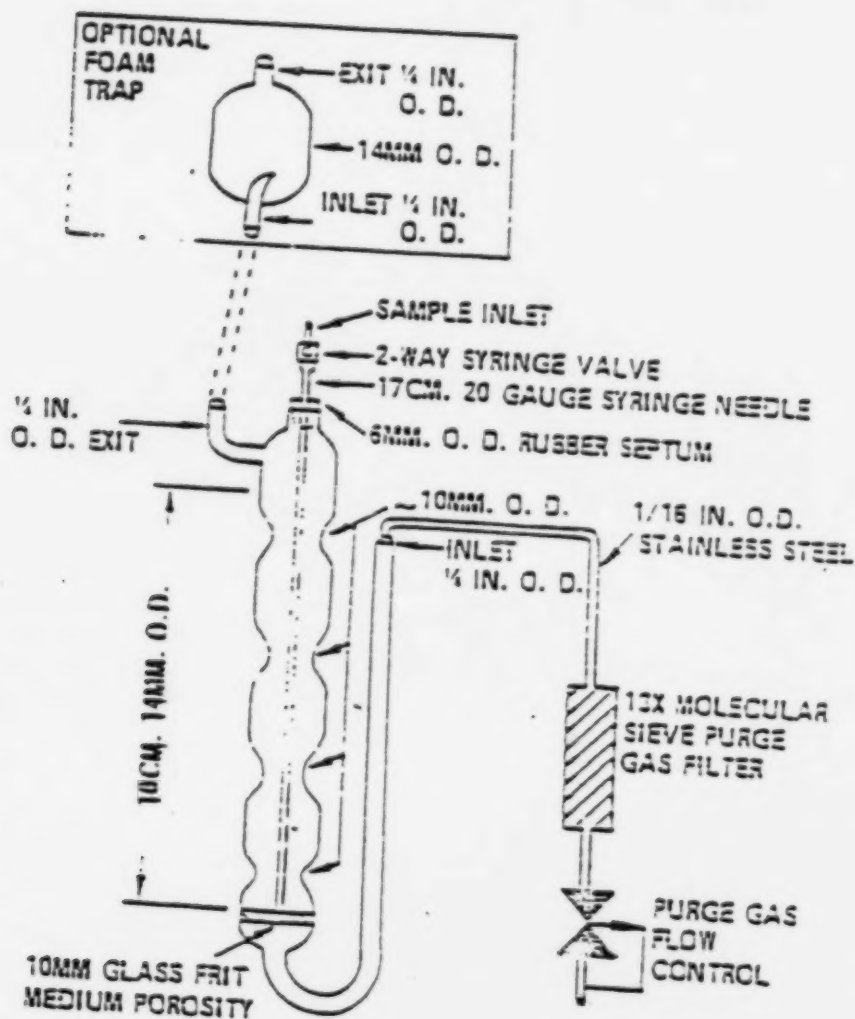


Figure 1. Purging device

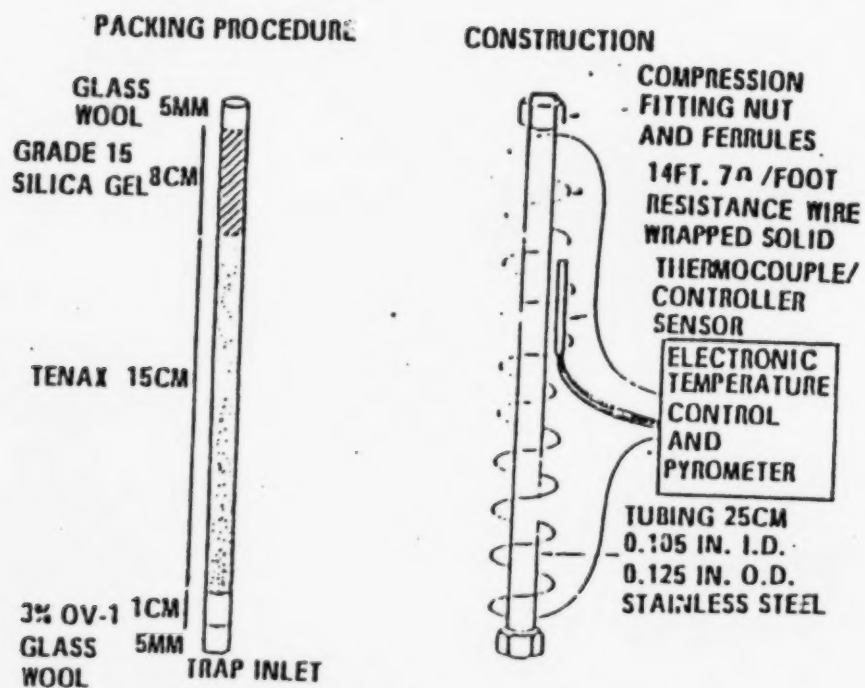


Figure 2. Trap packings and construction to include desorb capability

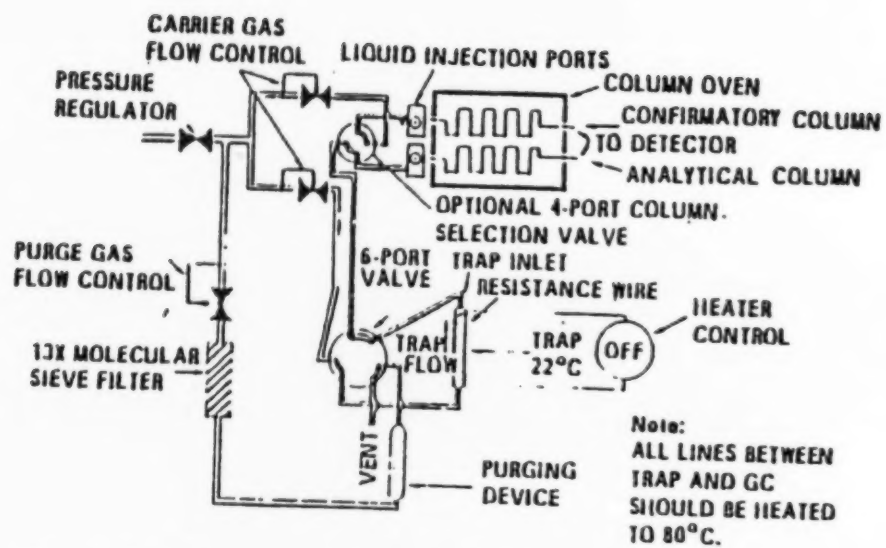


Figure 3. Schematic of purge and trap device - purge mode

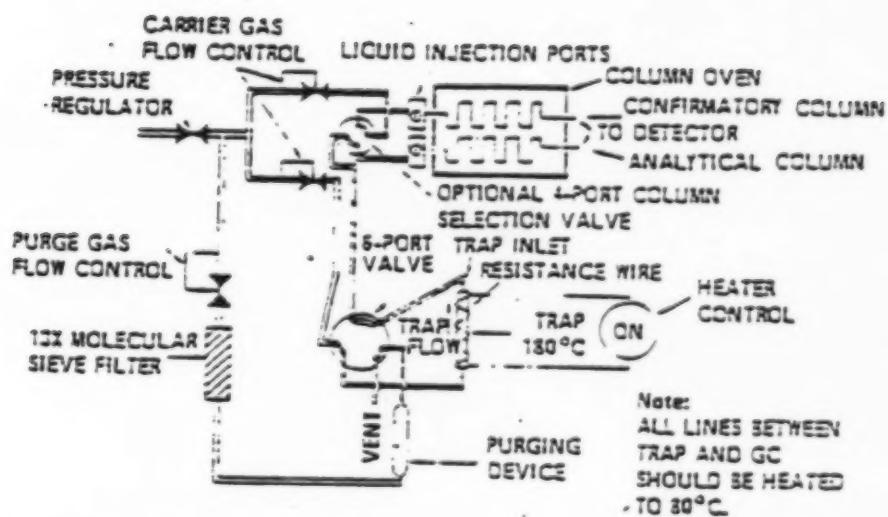


Figure 4. Schematic of purge and trap device - desorb mode

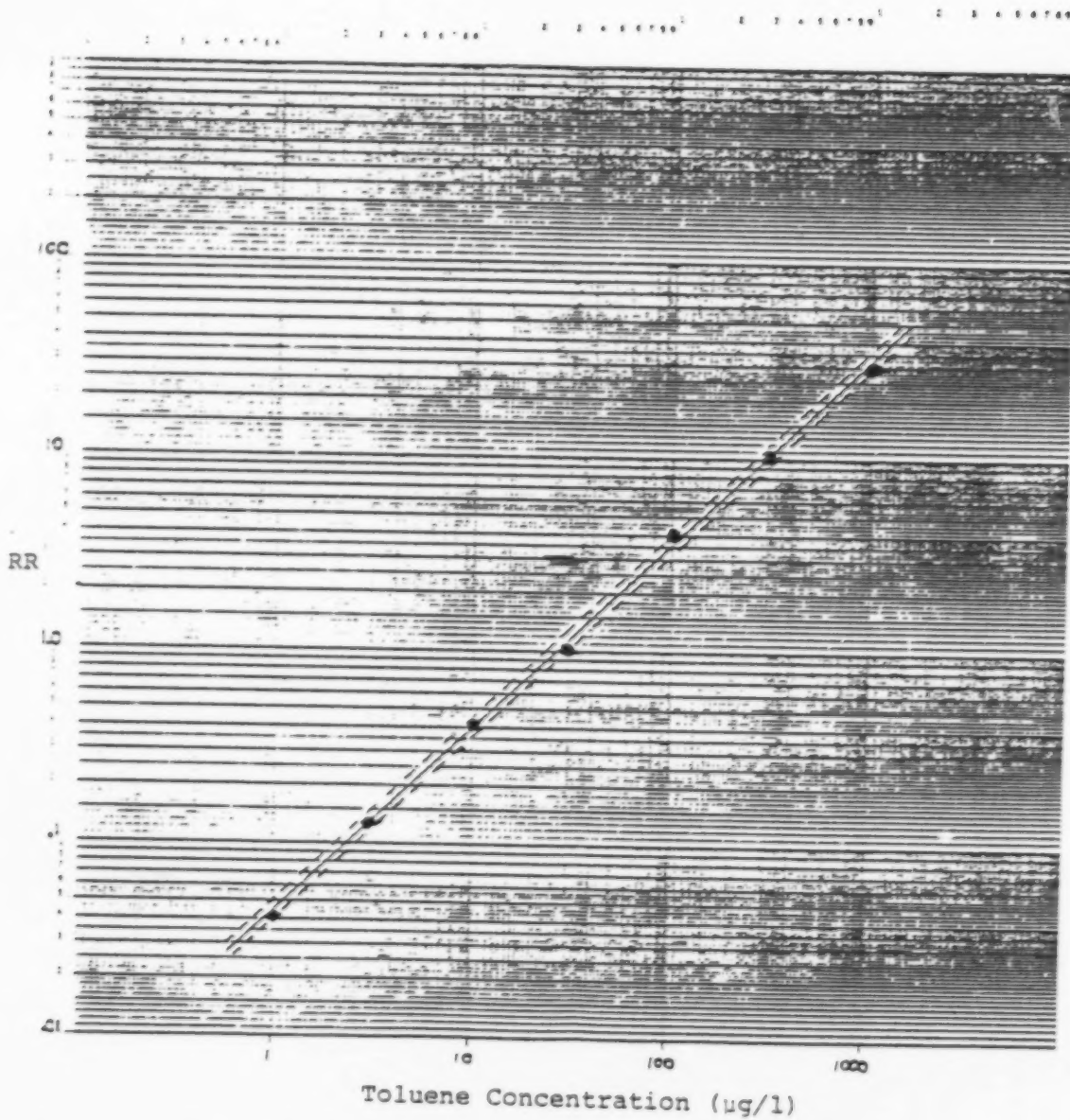


Figure 5.

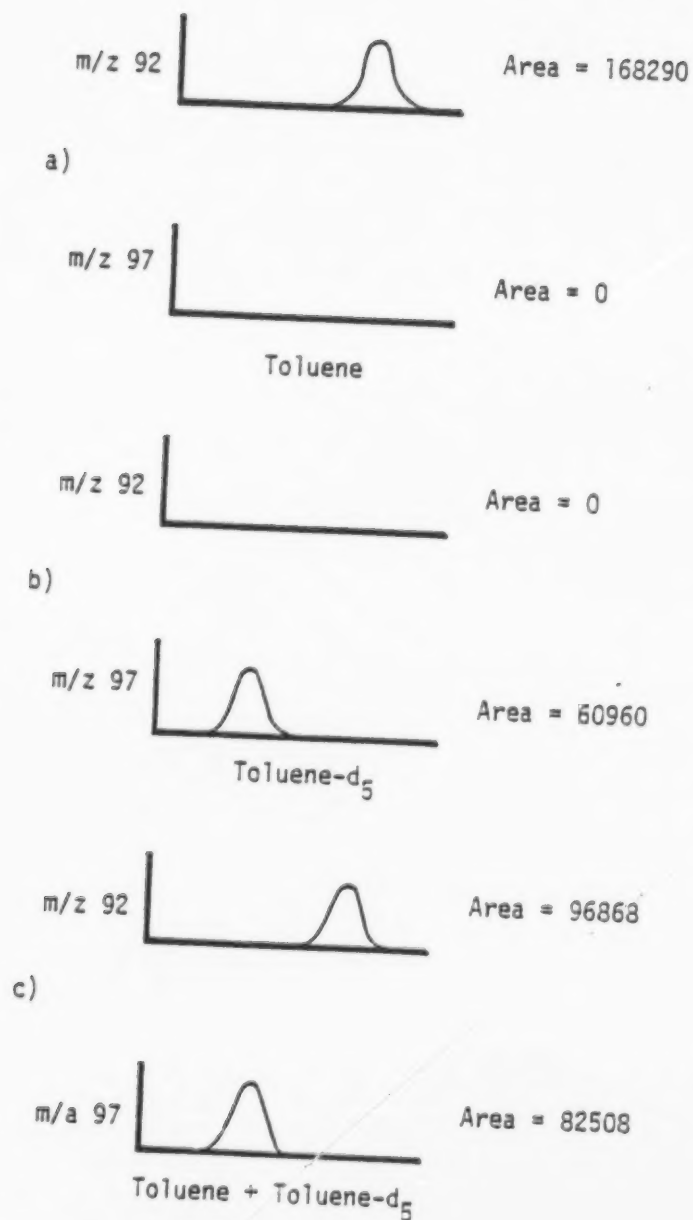


Figure 6. Extracted Ion Current Profiles for a) Toluene, b) Toluene-d₅, and Toluene plus Toluene-d₅.

COLUMN: 1% SP-1000 ON: CATERPAC-3
 PROGRAM: 45°C-3 MINUTES 8°/MINUTES TO 220°C

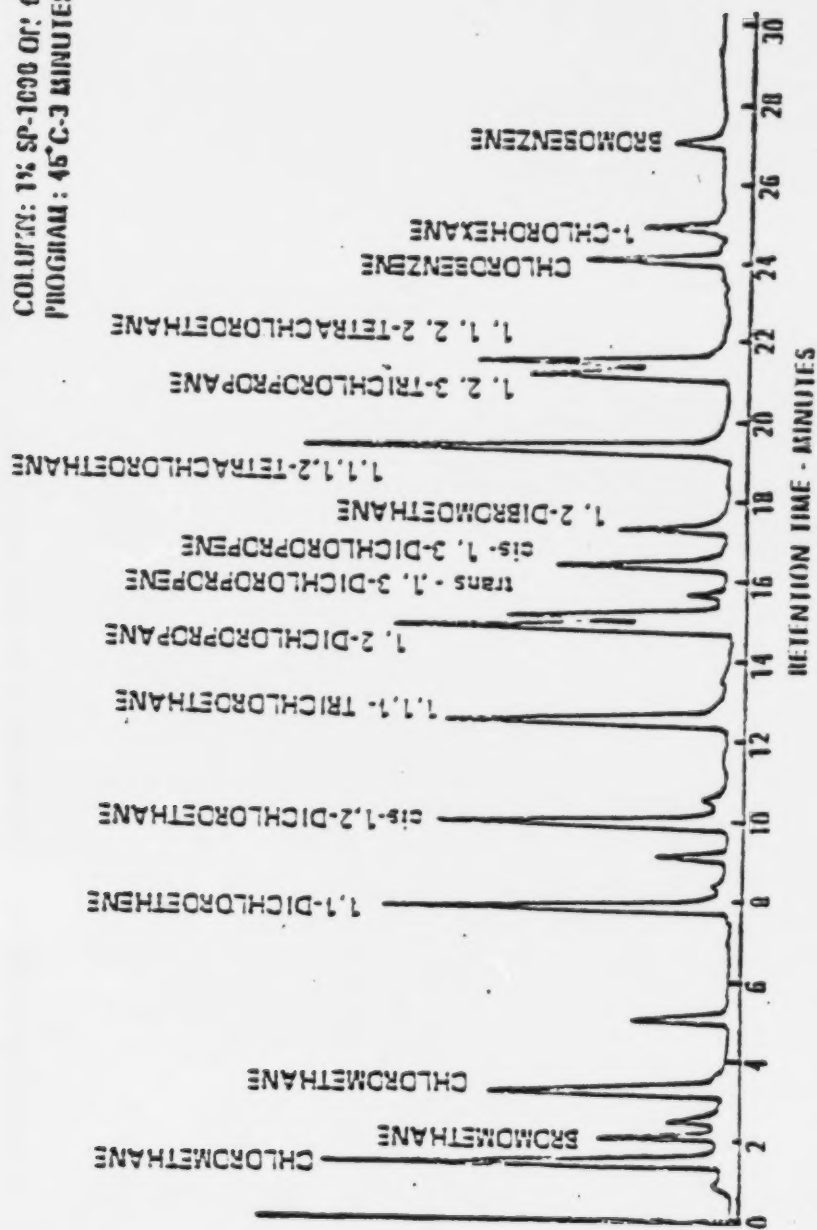


Figure 7. Gas chromatogram of volatile organics by purge and trap

APPENDIX C

INTERIM DRAFT - METHOD 1625 SEMIVOLATILE ORGANIC COMPOUNDS BY ISOTOPE DILUTION GC-MS

1. SCOPE AND APPLICATION

- 1.1 This method is designed to determine those "unambiguous priority pollutants," associated with the Consent Decree, that are solvent extractable and amenable to gas chromatography. The compounds listed in Tables 1 and 2 may be determined by this method.
- 1.2 This method is applicable to the determination of these compounds in municipal and industrial discharges. It is designed to be used to meet the survey requirements of the Effluent Guidelines Division (EGD) and the requirements of the National Pollutants Discharge Elimination System (NPDES).
- 1.3 The detection limit of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limits listed in Tables 3 and 4 represent the minimum quantity that must be injected into the system to get confirmation by the mass spectrometric method described below.
- 1.4 The GC-MS parts of this method are recommended for use only by analysts experienced in GC-MS or under the close supervision of such qualified persons.

2. SUMMARY OF METHOD

- 2.1 A 1-liter sample of wastewater is extracted, with methylene chloride using separatory funnel or continuous extraction techniques. If emulsions are a problem, continuous extraction techniques should be used. The extract is dried over sodium sulfate and concentrated to a volume of 1 ml using a Kuderna-Danish (K-D) evaporator. Chromatographic conditions are described which allow for the separation of the compounds in the extract.
- 2.2 Identification of a compound (qualitative analysis) is performed by an evaluation of background corrected characteristic ions.
- 2.3 Quantitative analysis is performed by GC-MS using isotope dilution.

3. INTERFERENCES

- 3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.
- 3.2 Interferences coextracted from the samples will vary considerably from source to source, depending upon the diversity of the industrial complex or municipality being sampled.

3.3 This analytical procedure may not have sufficient resolution to differentiate between certain isomeric pairs. These are anthracene and phenanthrene, chrysene and benzo(a)anthracene, and benzo(b)fluoranthene and benzo(k)fluoranthene. The GC retention time and mass spectral data are not sufficiently unique to make an unambiguous distinction between these compounds. Alternative techniques should be used to identify these specific compounds. See Reference 1.

4. APPARATUS AND MATERIALS

4.1 Sampling equipment, for discrete or composite sampling.

4.1.1 Sample bottle, glass, 1-liter minimum volume. The container must be washed and solvent rinsed before use to minimize interferences.

4.1.2 Bottle caps - threaded to fit sample bottles. Caps must be lined with Teflon. Aluminum foil may be substituted if the sample is not corrosive.

4.1.3 Compositing equipment - automatic or manual compositing system. Must incorporate glass sample containers for the collection of a minimum of 1 liter. Sample containers must be kept refrigerated during sampling. No plastic or rubber tubing other than Teflon may be used on the system.

4.2 Separatory funnel - 2000 ml, with Teflon stopcock.

4.3 Drying column - A 20-mm ID pyrex chromatographic column equipped with coarse glass frit or glass wool plug.

4.4 Kuderna-Danish (K-D) Apparatus

- 4.4.1 Concentrator tube - 10 ml, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked. Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.
 - 4.4.2 Evaporative flask - 500 ml (Kontes K-57001-0500 or equivalent). Attach to concentrator tube with springs (Kontes K-662750-0012).
 - 4.4.3 Snyder column - three-ball macro (Kontes K503000-0232 or equivalent).
 - 4.4.4 Snyder column - two-ball micro (Kontes K-569002-0219 or equivalent).
 - 4.4.5 Boiling chips-extracted, approximately 10/40 mesh.
- 4.5 Water bath - heated, with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 4.6 Gas chromatograph - analytical system complete with gas chromatograph capable of on-column injection and all required accessories including column supplies, gases, etc.
- 4.6.1 Column 1 - for Base/Neutral a 6-foot glass column (1/4 in OD x 2 mm ID) packed with 3 percent SP-2250 coated on 100/120 Supelcoport (or equivalent).
 - 4.6.2 Column 2 - for Acids, a 6-foot glass column (1/4 in OD x 2 mm ID) packed with 1 percent SP-1240 DA coated on 100/200 mesh Supelcoport (or equivalent).

- 4.7 Mass Spectrometer - capable of scanning from 42 to 450 a.m.u. every 3 seconds or less at 70 volts (nominal) and producing a recognizable mass spectrum (background corrected) at unit resolution from 50 ng of decafluorotriphenylphosphine (DFTPP) when the sample is introduced through the GC inlet (Reference 2). The mass spectrometer must be interfaced with a gas chromatograph equipped with an injector system designed for all-glass on-column packed column use. All sections of the transfer lines must be glass or glass-lined and must be deactivated. (Use Sylon-CT, Supelco, Inc., or equivalent to deactivate.) Note: Systems utilizing a jet separator for the GC effluent are recommended since membrane separators may lose sensitivity for light molecules and glass frit separators may inhibit the elution of polynuclear aromatics. Any of these separators may be used provided that it gives recognizable mass spectra (background corrected) and acceptable calibration points at the limit of detection specified for each individual compounds listed in Tables 3 and 4.
- 4.8 A computer system must be interfaced to the mass spectrometer to allow acquisition of continuous mass scans for the duration of the chromatographic program. The computer system should also be equipped with mass storage devices for saving all data from GC-MS runs. There must be computer software available to allow searching any GC-MS run for specific ions and plotting the intensity of the ions with respect to time or scan number. The ability to integrate the area under any specific ion plot peak is essential for quantification.
- 4.9 Continuous liquid-liquid extractors - Teflon or glass connecting joints and stopcocks, no lubrication (Hershberg-Wolf Extractor).

5. REAGENTS

- 5.1 Sodium hydroxide - (Reagent Grade) 6N in distilled water.
- 5.2 Sulfuric acid - (Reagent Grade) 6N in distilled water.
- 5.3 Sodium sulfate - (Reagent Grade) granular anhydrous (rinsed with methylene chloride (20 ml/g) and conditioned at 400°C for 4 hours).
- 5.4 Methylene chloride - distilled in glass or equivalent.
- 5.5 Stock standards - obtain stock standard solutions at a concentration of 1.00 $\mu\text{g}/\mu\text{l}$. For example, dissolve 0.100 grams of assayed reference material in a mixture of methylene chloride and benzene (9/1, v/v) and dilute to volume in a 100-ml ground glass stoppered volumetric flask. The stock solution is transferred to 15 ml Teflon-lined screw cap vials.
- 5.6 Labeled Compound Spiking Solutions - From stock standards prepared as above, prepare the labeled spiking solutions such that they contain 200 $\mu\text{g}/\text{ml}$ of the labeled compounds. Separate Acid and Base/Neutral solutions were required to prevent chemical reactions in solution.
- 5.7 All standard solutions should be stored in the dark at 4°C and should be checked for signs of degradation prior to use.

6. CALIBRATION STANDARDS

- 6.1 Prepare calibration standards that contain the compounds of interest. The standards should be prepared at concentrations that will completely bracket the working range of the chromatographic system (two or more orders of magnitude are suggested). If the limit of detection (Tables 3 or 4) can be calculated as 20 ng injected, for example, prepare standards at 1 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, etc. so that injections of 1-5 μl of the calibration standards will define the linearity of the detector in the working range.
- 6.2 Assemble the necessary gas chromatographic apparatus and establish operating conditions equivalent to those indicated in Tables 3 and 4. By injecting calibration standards, establish the linear range of the analytical system and demonstrate that the analytical system meets the limits of detection requirements of Tables 3 and 4. If the sample gives peak areas above the working range, dilute and reanalyze according to Method 625.
- 6.3 The isotope dilution approach is used for extractable organic compounds when appropriate labeled compounds are available and when interferences do not preclude the analysis. A calibration curve encompassing the concentration range of interest is prepared for each compound to be determined. When more than one order of magnitude in concentration is analyzed, plot relative response (RR) versus concentration ($\mu\text{g/ml}$) on log-log paper or use a logarithmically weighted linear regression. If a smaller concentration range is to be covered, linear plots and simple linear regressions are acceptable. An example of a calibration curve for phenol using phenol- d_5 is given in

Figure 1. Also shown in Figure 1 are the ± 10 percent error limits (dotted lines). Relative Response is determined according to the procedures described below. A minimum of three data points are required for calibration but more points are desirable.

6.3.1 The relative response (RR) of naturally abundant compound-to-enriched compound is determined from isotope ratio values calculated from acquired data. Three isotope ratios are used in this process:

R_x = the isotope ratio of pure naturally abundant compound,

R_y = the isotope ratio of pure enriched compound and,

R_m = the isotope ratio of the analytical mixture of naturally abundant and enriched compounds.

The correct way to calculate RR is:

$$RR = \frac{(R_y - R_m)(R_x + 1)}{(R_m - R_x)(R_y + 1)}$$

If R_m is not between $2R_y$ and $0.5R_x$, the method does not apply and the extract should be analyzed according to Method 625.

6.3.2 In most cases, the retention times of enriched and naturally abundant compounds are the same and isotope ratios (R's) can be calculated from the extracted ion current profiles where:

$$R = \frac{\text{Area at } m/z_1}{\text{Area at } m/z_2}$$

If either of the areas is zero, it is assigned a value of one in the calculations; that is if:

$$\begin{aligned} \text{area of } m/z_1 &= 50721 \text{ and} \\ \text{area of } m/z_2 &= 0 \end{aligned}$$

$$\text{then } R = \frac{50721}{1} = 50720$$

The m/z 's are always selected such that $R_x > R_y$.

When there is a difference in retention times (RT) between the naturally abundant and enriched compounds, some special precautions are required in order to determine the isotope ratios. First, R_x , R_y and R_m are defined as:

$$R_x = \frac{\text{Area } m/z_1 \text{ (at } RT_1\text{)}}{1}$$

$$R_y = \frac{1}{\text{Area } m/z_2 \text{ (at } RT_2\text{)}}$$

$$R_m = \frac{\text{Area } m/z_1 \text{ (at } RT_1\text{)}}{\text{Area } m/z_2 \text{ (at } RT_2\text{)}}$$

6.3.3 An example of the above calculations can be taken from the data plotted in Figure 2 for phenol and phenol- d_5 . For these data:

$$R_x = \frac{95807}{1} = 95810$$

$$R_y = \frac{1}{71760} = .00001394$$

and

$$R_m = \frac{35780}{27892} = 1.280$$

The RR for the above data is then calculated using the equation given in 6.3.1 for the example RR = 1.280. It should be noted that not all isotopically enriched compounds elute with their naturally abundant analogs.

7. QUALITY CONTROL

- 7.1 Before processing any samples, demonstrate through the analysis of a method blank that all glassware and reagents are interference-free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against chronic laboratory contamination.
- 7.2 Standard quality assurance practices should be used with this method. Field replicates should be collected to determine the precision of the sampling technique. Laboratory replicates should be analyzed to determine the precision of

the analysis. Fortified samples should be analyzed to determine the accuracy of the analysis. Field blanks should be analyzed to check for contamination introduced during sampling and transportation.

7.3 Difluorobiphenyl, which is added to the sample extract just prior to injection into the GC/MS, is used as an instrument check. The response and retention time of this compound are monitored for changes as an indicated of instrument performance. Response and retention time should be recorded in the QC log.

8. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

8.1 Samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of Tygon tubing and other potential sources of contamination.

8.2 The samples must be iced or refrigerated from the time of collection until extraction. Chemical preservatives should not be used.

8.3 All sample extractions must be initiated within 48 hours from receipt by the laboratory and completely analyzed within thirty days of collection.

9. SAMPLE EXTRACTION (BASE/NEUTRALS AND ACIDS)

- 9.1 Measure out $1.00 \pm .01$ liters of sample and pour the entire sample into a two-liter separatory funnel. Add labeled compounds for use with isotope dilution quantitation. Labeled compounds should be added at concentrations of $100 \mu\text{g/l}$ and an interval of one to two hours should pass between the addition and the beginning of the extraction. Adjust the pH of the sample with 6N NaOH to 11 or greater. Use multirange pH paper for the measurements. Proceed to Section 10 if continuous extraction is used.
- 9.2 Add 60 ml methylene chloride to the empty sample bottle, cap, and shake the bottle and contents 30 seconds to rinse the walls. Transfer the solvent into the separatory funnel, and extract the sample by shaking the funnel for two minutes with periodic venting to release excess vapor pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. If the emulsion cannot be broken, that is, recovery of less than 80 percent of the added solvent corrected for the water solubility of methylene chloride, use continuous extraction. Collect the methylene chloride extract in a 250-ml Erlenmeyer flask.
- 9.3 Add a second 60-ml volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

- 9.4 Perform a third extraction in the same manner. Pour the combined extract through a drying column containing three to four inches of anhydrous sodium sulfate, and collect it in a 500-ml K-D flask equipped with 10-ml concentrator tube. Rinse the Erlenmeyer flask with 20 to 40 ml of methylene chloride. Pour this through the drying column. Seal, label as base/neutral fraction, and proceed with the acid extraction.
- 9.5 Acid extraction - Adjust pH of the water, previously extracted for Base/Neutrals, with 6N H_2SO_4 to 2 or less. Serially extract with 60, 60, and 60 ml portions of distilled-in-glass methylene chloride. Collect and combine the acid extracts in a 250-ml Erlenmeyer flask then dry the extracts by passing them through a column of anhydrous sodium sulfate. Rinse the Erlenmeyer flask with 20 to 40 ml of methylene chloride and pour through the drying column. Seal and label Acid fraction and prepare for concentration.
- 9.6 Concentrate the extracts (Base/Neutrals and Acids) in separate 500-ml K-D flasks equipped with 10-ml concentrator tubes.
- 9.7 Add 1 to 2 clean boiling chips to the flask and attach a three-ball macro-Snyder column. Prewet the Snyder column by adding about 1-ml methylene chloride through the top. Place the K-D apparatus in a steam bath. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column actively chatter but the chambers do not flood. When the liquid has reached an apparent volume 1 ml, remove the K-D apparatus and allow the solvent to drain for at least 10

minutes while cooling. Remove the Snyder column and rinse the flask and its lower joint in the concentrator tube with 1 to 2 ml of methylene chloride. A 5-ml syringe is recommended for this operation.

9.8 Add a clean boiling chip and attach a two-ball micro-Snyder column to the concentrator tube in 9.7. Prewet the column by adding about 0.5-ml methylene chloride through the top. Place the K-D apparatus in a steam bath. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5-10 minutes. At the proper rate of distillation, the balls of the column actively chatter but the chambers do not flood. When the liquid reaches an apparent volume of about 0.5 ml, remove the K-D apparatus from the water bath and allow the solvent to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with approximately 0.2 ml of methylene chloride. Adjust the final volume to 1.0 ml, seal and label the container.

10. EMULSIONS/CONTINUOUS EXTRACTION

10.1 Place 100 to 150 ml of methylene chloride in the extractor and 200 to 500 ml of methylene chloride in the distilling flask.

10.2 Add the aqueous sample (pH 12 to 13) to the extractor. Check and adjust the pH of the sample after 1-2 hours when a continuous extractor is used. Extract for 24 hours. Remove the distilling flask and pour the contents through a drying column containing 7 to 10 cm of anhydrous sodium sulfate. Collect the methylene chloride in a 500-ml K-D evaporator flask equipped with a 10-ml concentrator tube. Seal, label as the Base/Neutral fraction, and concentrate as per section 9.7 to 9.8.

10.3 Adjust the pH of the sample in the continuous extractor to 2 or below using 6N sulfuric acid. Charge a clean distilling flask with 500 ml of methylene chloride. Extract for 24 hours. Remove the distilling flask and pour the contents through a drying column containing 7 top 10 cm of anhydrous sodium sulfate. Collect the methylene chloride layer on a K-D evaporator flask equipped with a 10-ml concentrator tube. Label as the Acid fraction. Concentrate as per Sections 9.7 and 9.8.

11. CALIBRATION OF THE GC-MS SYSTEM

11.1 At the beginning of each day, the calibration of the GC-MS system must be checked and adjusted if necessary to meet DFTPP specifications (11.3). Each day base-neutrals are measured, the column performance specification (12.1) with benzidine must be met. Each day the acids are measured, the column performance specification (13.1) with pentachlorophenol must be met. DFTPP can be mixed in solution with either of these compounds to complete two specifications with one injection, if desired.

11.2 To perform the calibration test of the GC-MS system, the following instrumental parameters are required.

Electron energy - 70 volts (nominal).

Mass range - 42 to 450 a.m.u.

Scan time - 3 seconds or less.

11.3 GC-MS system calibration - Evaluate the system performance each day that it is to be used for the analysis of samples or blanks by examining the mass spectrum of DFTPP. Inject a solution containing 50 ng DFTPP and check to ensure that

performance criteria listed in Table 2 are met. If the system performance criteria are not met, the analyst must retune the spectrometer and repeat the performance check. The performance criteria must be met before any samples or standards may be analyzed.

12. GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF BASE/NEUTRAL FRACTION

- 12.1 At the beginning of each day that base/neutral analyses are to be performed, inject 100 nanograms of benzidine either separately or as part of a standard mixture that may also contain 50 ng of DFTPP. Acceptable performance is achieved when the benzidine is identified according to Section 14.
- 12.2 Establish chromatographic conditions equivalent to those in Table 3. Included in this table are estimated retention times and sensitivities that can be achieved by this method. Examples of the separations achieved by these columns are shown in Figure 3.
- 12.3 Program the GC-MS to operate in the full spectrum scanning mode.
- 12.4 Add difluorobiphenyl to the extract at 20 $\mu\text{g/ml}$ just prior to injection into the GC/MS.
- 12.5 Inject 2 to 5 μl of the sample extract. The solvent-flush technique is preferred. If the response for any ion exceeds the linear range of the system, dilute the extract and re-analyze according to Method 625.
- 12.6 Qualitative and quantitative measurements are made as described in Sections 14 and 15. When the extracts are not being used for analysis, store them in vials with unpierced septa in the dark at 4°C.

13. GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF ACID FRACTION

- 13.1 At the beginning of each day that acid fraction analyses are to be performed, inject 50 nanograms of pentachlorophenol either separately or as part of a standard mixture that may also contain DFTPP. Acceptable performance is achieved when pentachlorophenol is identified according to Section 14.
- 13.2 Establish chromatographic conditions equivalent to those in Table 4. Included in this table are estimated retention times and sensitivities that can be achieved by this method. An example of the separation achieved by the column is shown in Figure 4.
- 13.3 Program the GC-MS to operate in the full spectrum scanning mode.
- 13.4 Add difluorobiphenyl to the extract at 20 $\mu\text{g/ml}$ just prior to injection into the GC/MS.
- 13.5 Inject 2 to 5 μl of the sample extract. The solvent-flush technique is preferred. If the response for any ion exceeds the linear range of the system, dilute the extract and reanalyze.
- 13.6 Qualitative and quantitative measurements are made as described in Sections 14 and 15. When the extracts are not being used for analysis, store them in vials with unpierced septa in the dark at 4°C.

14. MASS SPECTROMETRY

14.1 Naturally Abundance Compounds - Identification of naturally abundant compounds is accomplished by (1) comparison of extracted ion current profile (EICP) areas with those of known standards and (2) evaluation of the retention time difference between the naturally abundant compound and its labeled analog.

14.1.1 EICP areas for the characteristic masses given in Tables 8 and 9 must agree within $\pm 20\%$ with those of a known standard. If a spectral interference is suspected, additional masses should be investigated.

14.1.2 The retention time difference between the naturally abundant compound and its labeled analog must agree within ± 6 seconds with those of known standards.

14.2 Labeled Compounds - Identification of labeled compounds is accomplished by (1) comparison of EICP areas with those of known standards and (2) evaluation of retention times.

14.2.1 EICP areas for the characteristic masses given in Tables 8 and 9 must agree within $\pm 20\%$ with those of a known standard. If a spectral interference is suspected, additional masses should be investigated.

14.2.2 The retention time of the labeled compound must agree within ± 60 seconds with those of a known standard.

15. CALCULATIONS

15.1 When a compound has been identified, the quantification of that compound is based on integrated areas from the extracted ion current profiles. If the sample produces an interference for the primary (first listed) pair of ions, use a secondary pair to quantify.

15.2 Quantitative Calculations - By adding a known amount of each isotopically enriched compound to be analyzed to every sample prior to extraction, automatic correction for component recovery is accomplished. This is because the enriched and naturally abundant compounds will exhibit the same matrix effects during sample preparation. Relative response (RR) values for sample mixtures are used in conjunction with the calibration curves described in Section 6.3 to determine concentrations directly as long as the isotopically enriched compound spiking levels are constant. For the phenol example given in Figure 2, RR would be equal to 1.283. For this RR value, the phenol calibration curve given in Figure 1 indicates a concentration of 108 $\mu\text{g/ml}$. This value is then multiplied by the concentration factor (final volume of extract/initial volume of water sample) to get the original sample concentration in $\mu\text{g/l}$.

15.2.1 For isotope dilution analyses, the primary pairs of m/z 's for quantitation are given in Table 3. If there are interferences at these m/z 's or if an alternative labeled compound is employed, the best pair of m/z 's can be predicted by calculating an ion selection factor (ISF) (see Reference 4) where:

$$ISF = \log \frac{R_x}{R_y} - \log \frac{200}{a_x + b_y}$$

R_x and R_y are defined in Section 6.5.1 and a_x and b_y are the percent of total ionization values for the more abundant m/z 's of the natural and enriched compounds, respectively. Generally, the best m/z 's are found among the more abundant m/z 's in the spectra. Some ISF's for 1,2-dichlorobenzene and 1,2-dichlorobenzene- d_4 are given below.

m/z 's	R_x	R_y	a_x	b_y	ISF
146/152	33247	3.23×10^{-5}	23.9	15.0	8.30
148/152	17874	3.23×10^{-5}	12.9	15.0	7.89
111/115	14063	4.58×10^{-5}	10.3	10.3	7.50
146/150	15.7	2.10×10^{-5}	23.9	22.4	5.24
148/150	8.44	2.71×10^{-5}	12.9	22.4	4.74
75/78	17851	0.151	12.3	9.37	4.11

For these two compounds, isotope ratios made a m/z 's 146 and 152 should be the best.

16. ACCURACY AND PRECISION

16.1 Precision of the method is determined by preparing and analyzing replicate sample aliquots. Accuracy of the method is determined by analyzing samples spiked with the compounds of interest.

16.2 See Reference 5.

17. REFERENCES

1. Method 610, Polynuclear Aromatic Hydrocarbons, U.S. EPA, EMSL, Cincinnati, Ohio 45268, 1979.
2. "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography-Mass Spectrometry Systems," J. W. Eichelberger, L. E. Harris and W. L. Budde, Anal. Chem., **47**, 995-1000 (1975)
3. "Quality Assurance and Quality Control Procedures for Screening and Verification of Industrial Effluents for Priority Pollutants," U.S. EPA, EMSL, Cincinnati, Ohio 45268, 1979.
4. "A Fundamental Approach to Measurement Parameter Selection for Quantitative Isotope Dilution GC/MS Determinations," B. N. Colby, A. E. Rosecrance and M. E. Colby, submitted for publication in Analytical Chemistry.
5. "Isotope Dilution Gas Chromatography-Mass Spectrometry for the Determination of Priority Pollutants in Industrial Effluents," B. N. Colby, R. G. Beimer, D. R. Rushneck, and W. A. Telliard, submitted for publication in Analytical Chemistry.

TABLE 1
BASE/NEUTRAL EXTRACTABLE COMPOUNDS

<u>Compound</u>	<u>STORET No.</u>
Acenaphthene	34205
Acenaphthylene	34200
Anthracene	34220
Benzo(a)anthracene	34525
Benzo(b)fluoranthene	34230
Benzo(k)fluoranthene	34242
Benzo(a)pyrene	34247
Benzo(ghi)perylene	34521
Benzidine	39120
Bis(2-chloroethyl)ether	34273
Bis(2-chloroethoxy)methane	34278
Bis(2-ethylhexyl)phthalate	39100
Bis(2-chloroisopropyl)ether	34283
4-Bromophenyl phenyl ether	34536
Butyl benzyl phthalate	34292
2-Chloronaphthalene	34581
4-Chlorophenyl phenyl ether	34641
Chrysene	34320
Dibenzo(a,h)anthracene	34556
Di-n-butylphthalate	39110
1,3-Dichlorobenzene	34556
1,4-Dichlorobenzene	34571
1,2-Dichlorobenzene	34536
3,3'-Dichlorobenzidine	34631
Diethylphthalate	34336
Dimethylphthalate	34341
2,4-Dinitrotoluene	34611
2,6-Dinitrotoluene	34625
Dioctylphthalate	34536
1,2-Diphenylhydrazine	34346
Fluoranthene	34376
Fluorene	34381
Hexachlorobenzene	39700
Hexachlorobutadiene	34391
Hexachloroethane	34396
Hexachlorocyclopentadiene	34386
Indeno(1,2,3-cd)pyrene	34403
Isophorone	34408
Naphthalene	34536
Nitrobenzene	34417
N-nitrosodimethylamine	34438
N-nitrosodi-n-propylamine	34428
N-nitrosodiphenylamine	34433
Phenanthrene	34461
Pyrene	34463
2,3,7,8-Tetrachlorodibenzo-p-dioxin	34673
1,2,4-Trichlorobenzene	34531

TABLE 2
ACID EXTRACTABLE COMPOUNDS

<u>Compound</u>	<u>Starst No.</u>
4-Chloro-3-methylphenol	34452
2-Chlorophenol	34526
2,4-Dichlorophenol	34601
2,4-Dimethylphenol	34606
2,4-Dinitrophenol	34616
2-Methyl-4,6-dinitrophenol	34657
2-Nitrophenol	34591
4-Nitrophenol	34646
Pentachlorophenol	39094
Phenol	34694
2,4,6-Trichlorophenol	34621

Table 3

GAS CHROMATOGRAPHY OF BASE/NEUTRAL EXTRACTABLE COMPOUNDS

Compound	Retention Time (min.)	Limit of Detection	
		ng injected	ug/l
1,3-Dichlorobenzene	7.4	20	10
1,4-Dichlorobenzene	7.0	20	10
Hexachloroethane	0.4	20	10
Bis(2-chloroethyl) ether	0.4	20	10
1,2-Dichlorobenzene	0.4	20	10
Bis(methyl-2-chloroethyl) ether	0.4	20	10
N-nitroso-di-n-propyl amine	9.3	20	10
Nitrobenzene	-	20	10
Hexachlorobutadiene	11.1	20	10
1,2,4-Trichlorobenzene	11.4	20	10
Isophorone	11.6	20	10
Naphthalene	11.9	20	10
Bis(2-chloroethoxy) methane	12.1	20	10
Hexachlorocyclopentadiene	12.2	20	10
2-Chloronaphthalene	13.9	20	10
Acenaphthylene	15.9	20	10
Acenaphthene	17.4	20	10
Dimethyl phthalate	17.0	20	10
2,6-Dinitrotoluene	10.3	20	10
Fluorene	10.7	20	10
4-Chlorophenyl phenyl ether	19.5	20	10
2,4-Dinitrotoluene	19.5	20	10
1,2-Diphenyl hydrazine*	19.0	20	10
Diethyl phthalate	20.1	20	10
N-nitrosodiphenyl amine**	20.1	20	10
Hexachlorobenzene	20.5	20	10
4-Bromophenyl phenyl ether	21.0	20	10
Phenanthrene	21.2	20	10
Anthracene	22.0	20	10
Di-n-butyl phthalate	22.0	20	10
	24.7	20	10



TABLE 3 (Cont Inued)

Compound	Retention Time (Min)	Limit of Detection (ng Injected)	ug/l
Fluoranthene	26.5	20	10
Pyrene	27.3	20	10
Benzidine	28.0	20	10
Butyl benzyl phthalate	29.9	20	10
Bis(2-ethylhexyl) phthalate	30.6	20	10
Chrysene	31.5	20	10
Benzo(a)anthracene	31.5	20	10
3,3'-Dichlorobenzidine	32.2	20	10
Di-n-octyl phthalate	32.5	20	10
Benzo(b)fluoranthene	34.9	20	10
Benzo(k)fluoranthene	34.9	20	10
Benzo(a)pyrene	36.4	20	10
Indeno(1,2,3-cd)pyrene	42.7	50	25
Dibenz(a,h)anthracene	43.2	50	25
Benzo(ghi)perylene	45.1	50	25
N-Nitrosodimethylaniline	-	-	-
Bis(chloromethyl)ether	-	-	-
2,3,7,8-Tetrachlorodibenzo-p-dioxin	-	-	-

Six foot glass column (1/4 in. OD x 2 mm ID) packed with 3% SP-2250 coated on 100/120 mesh Supelcoport. Carrier gas: helium at 30 ml per min. Temperature program: Isothermal for 4 minutes at 50°C, then 80 per min to 270°C. Hold at 270°C for 30 minutes.

This is a minimum level at which the entire analytical system must give recognizable mass spectra (background corrected) and acceptable calibration points. Nanograms injected is based on a 2 ul injection of a one liter sample that has been extracted and concentrated to a volume of 1.0 ml.

* Detected as azobenzene

* Detected as diphenylamine.

Table 4
GAS CHROMATOGRAPHY OF ACID EXTRACTABLE COMPOUNDS

Compound	Retention Time* (Min)	Limit of Detection#	
		ng injected	µg
2-Chlorophenol	5.9	50	25
2-Nitrophenol	6.4	50	25
Phenol	8.0	50	25
2,4-Dimethylphenol	9.4	500	250
2,4-Dichlorophenol	9.8	50	25
2,4,6-Trichlorophenol	11.8	50	25
4-Chloro-3-methylphenol	13.2	50	25
2,4-Dinitrophenol	15.9	500	250
2-Methyl-4,6-dinitrophenol	16.2	500	250
Pentachlorophenol	17.5	250	125
4-Nitrophenol	20.3	50	25

*Six-foot glass column (1/4 in. OD x 2 mm ID) packed with 1 percent SP-1240 DA coated on 100/120 mesh Supelcoport. Carrier gas: helium at 30 ml per min. Temperature program: 2 min. isothermal at 70°, then 8° per min to 200°C.

#This is a minimum level at which the entire analytical system must give recognizable mass spectra (background corrected) and acceptable calibration points. Nanograms injected is based on a 2 µl injection of a one-liter sample that has been extracted and concentrated to 1.0 ml.

Table 5

BASE/NEUTRAL EXTRACTABLE ISOTOPE DILUTION COMPOUND ANALOGS AND IONS

<u>Compound</u>	<u>Labeled Analog</u>	<u>Primary m/z's</u>
1,3-Dichlorobenzene	*	*
1,4-Dichlorobenzene	1,4-dichlorobenzene, d ₄	146/152
Hexachloroethane	*	*
Bis(2-chloroethyl) ether	*	*
1,2-Dichlorobenzene	1,2-dichlorobenzene-d ₄	146/152
Bis(methyl-2-chloroethyl) ether	*	*
N-Nitrosodipropyl amine	*	*
Isophorone	*	*
Nitrobenzene	Nitrobenzene-d ₅	123/128
Hexachlorobutadiene	*	*
1,2,4-Trichlorobenzene	*	*
Naphthalene	Naphthalene-d ₈	128/136
Bis(2-chloroethoxy) methane	*	*
Hexachlorocyclopentadiene	*	*
2-Chloronaphthalene	*	*
Acenaphthylene	*	*
Acenaphthene	Acenaphthene, d ₁₀	154/164
Dimethyl phthalate	*	*
2,6-Dinitrotoluene	*	*
Fluorene	Fluorene, d ₁₀	166/176
4-Chlorophenyl phenyl ether	4-Chlorophenyl phenyl ether, d ₅	204/209
2,4-Dinitrotoluene	*	*
1,2-Diphenylhydrazine**	*	*
Diethylphthalate	*	*
N-Nitrosodiphenylamine***	N-Nitrosodiphenylamine, d ₅	169/175
Hexachlorobenzene	*	*
4-Bromophenyl phenyl ether	*	*
Phenanthrene	Phenanthrene, d ₁₀	178/188
Anthracene	Anthracene, d ₁₀	178/188
Dibutyl phthalate	*	*
Fluoranthene	*	*
Pyrene	Pyrene, d ₁₀	202/212
Benzidine	*	*
Butyl benzyl phthalate	*	*
Bis(2-ethyl hexyl) phthalate	*	*
Chrysene	Chrysene-d ₁₂	228/240
Benzo(a)anthracene	*	*
3,3'-Dichlorobenzidine	*	*
Dioctyl phthalate	Dioctyl phthalate, d ₄	149/153
Benzo(b)fluoranthene	*	*
Benzo(k)fluoranthene	*	*
Benzo(a)pyrene	Benzo(a)pyrene-d ₁₂	252/264
Indeno(1,2,3-c,d)pyrene	*	*
Dibenzo(a,h)anthracene	*	*
Benzo(g,h,i)perylene	*	*
N-nitrosodimethyl amine	*	*
Bis(chloromethyl) ether	*	*
2,3,7,8-Tetrachlorodibenzo-p-dioxin	*	*

*Data not available at time of writing. **Detected as azobenzene

***Detected as diphenylamine

Table 6
ACID EXTRACTABLE ISOTOPE DILUTION COMPOUND ANALOGS AND IONS

<u>Compound</u>	<u>Labeled Analog</u>	<u>m/z's</u>
2-Chlorophenol	2-Chlorophenol-d ₄	128/132
2-Nitrophenol	2-Nitrophenol-d ₄	139/143
Phenol	Phenol-d ₅	94/99
2,4-Dimethylphenol	2,4-Dimethylphenol-d ₃	122/125
2,4-Dichlorophenol	2,4-Dichlorophenol-d ₃	162/167
2,4,6-Trichlorophenol	2,4,6-Trichlorophenol-d ₂	196/200
4-Chloro-3-methyl phenol	*	*
2,4-Dinitrophenol	2,4-Dinitrophenol-d ₃	184/187
2-Methyl-4,6-dinitrophenol	*	*
Pentachlorophenol	Pentachlorophenol- ¹³ C ₆	266/272
4-Nitrophenol	*	*

*Data not available at time of writing.

Table 7
DFTPP KEY IONS AND ION ABUNDANCE CRITERIA

Mass	Ion Abundance Criteria
51	30 to 60 percent of mass 198
68	Less than 2 percent of mass 69
70	Less than 2 percent of mass 69
127	40 to 60 percent of mass 198
197	Less than 1 percent of mass 198
198	Base peak, 100 percent relative abundance
199	5 to 9 percent of mass 198
275	10 to 30 percent of mass 198
365	Greater than 1 percent of mass 198
441	Present but less than mass 443
442	Greater than 40 percent of mass 198
443	17 to 23 percent of mass 442

Table 8

BASE/NEUTRAL EXTRACTABLE COMPOUND CHARACTERISTIC IONS

Compound	Characteristic Ions			Labeled Analog	Labeled Analog Characteristic Ions		
1,3-Dichlorobenzene	146	148	113	(1)	(1)		
1,4-Dichlorobenzene	146	148	113	d ₄	152	154	115
Hexachloroethane	117	201	199	(1)	(1)		
Bis(2-chloroethyl) ether	93	63	95	(1)	(1)		
1,2-Dichlorobenzene	146	148	113	d ₄	152	154	115
Bis(methyl-2-chloroethyl) ether	45	77	79	(1)	(1)		
N-Nitrosodipropyl amine	130	42	101	(1)	(1)		
Isophorone	82	95	138	(1)	(1)		
Nitrobenzene	77	123	65	d ₅	82	54	128
Hexachlorobutadiene	225	223	227	(1)	(1)		
1,2,4-Trichlorobenzene	180	182	109	(1)	(1)		
Naphthalene	128	129	127	d ₈	136	108	137
Bis(2-chloroethoxy) methane	93	95	123	(1)	(1)		
Hexachlorocyclopentadiene	237	235	272	(1)	(1)		
2-Chloronaphthalene	162	164	127	(1)	(1)		
Acenaphthylene	152	151	153	(1)	(1)		
Acenaphthene	154	153	152	d ₁₀	164	162	160
Dimethyl phthalate	163	194	164	(1)	(1)		
2,6-Dinitrotoluene	165	63	121	(1)	(1)		
Fluorene	166	165	167	d ₁₀	176	146	174
4-Chlorophenyl phenyl ether	204	206	141	d ₅	209	174	146
2,4-Dinitrotoluene	165	63	182	(1)	(1)		
1,2-Diphenylhydrazine*	77	93	105	(1)	(1)		
Diethylphthalate	149	177	150	(1)	(1)		
N-Nitrosodiphenylamine**	169	168	167	d ₆	175	173	53
Hexachlorobenzene	284	142	249	(1)	(1)		
4-Bromophenyl phenyl ether	248	250	141	(1)	(1)		
Phenanthrene	178	179	176	d ₁₀	188	160	184
Anthracene	178	179	176	d ₁₀	188	160	184
Dibutyl phthalate	149	150	104	(1)	(1)		

Table 8 (Continued)

Compound	Characteristic Ions			Labeled Analog	Labeled Analog Characteristic Ions		
Fluoranthene	202	101	100	(1)	(1)		
Pyrene	202	101	100	d ₁₀	212	208	211
Benzydine	184	92	185	(1)	(1)		
Butyl benzyl phthalate	149	91		(1)	(1)		
Bis(2-ethyl hexyl) phthalate	149	167	279	(1)	(1)		
Chrysene	228	226	229	d ₁₂	240	241	236
Benzo(a)anthracene	228	229	226	d ₁₂	240	241	236
3,3'-Dichlorobenzidine	252	254	126	(1)	(1)		
Dioctyl phthalate	149			d ₄	153		
Benzo(b)fluoranthene	252	253	125	(1)	(1)		
Benzo(k)fluoranthene	252	253	125	(1)	(1)		
Benzo(a)pyrene	252	253	125	d ₁₂	264	260	265
Indeno(1,2,3-c,d)pyrene	276	138	277	(1)	(1)		
Dibenzo(a,h)anthracene	278	139	279	(1)	(1)		
Benzo(g,h,i)perylene	276	138	277	(1)	(1)		
N-nitrosodimethyl amine	42	74	44	(1)	(1)		
Bis(chloromethyl) ether	45	49	51	(1)	(1)		
2,3,7,8-Tetrachlorodibenzo-p-dioxin	322	320	59	(1)	(1)		

*Detected as azobenzene

**Detected as diphenylamine

(1) Data not available at this time.

Table 9
ACID EXTRACTABLE COMPOUND CHARACTERISTIC IONS

Compound	Characteristic Ions			Labeled Analog	Labeled Analog Characteristic Ions		
2-Chlorophenol	128	64	130	d ₄	134	68	136
2-Nitrophenol	139	65	109	d ₄	143	69	113
Phenol	94	65	66	d ₅	99	71	70
2,4-Dimethylphenol	122	107	121	d ₃	125	110	124
2,4-Dichlorophenol	162	164	98	d ₃	165	167	101
2,4,6-Trichlorophenol	196	198	200	d ₂	202	101	136
4-Chloro-3-methyl phenol	142	107	144	*	*		
2,4-Dinitrophenol	184	63	154	d ₃	187	66	157
2-Methyl-4,6-dinitrophenol	198	182	77	*	*		
Pentachlorophenol	266	264	268	¹³ C ₆	171	173	175
4-Nitrophenol	65	139	109	*	*		

*Data not available at this time.

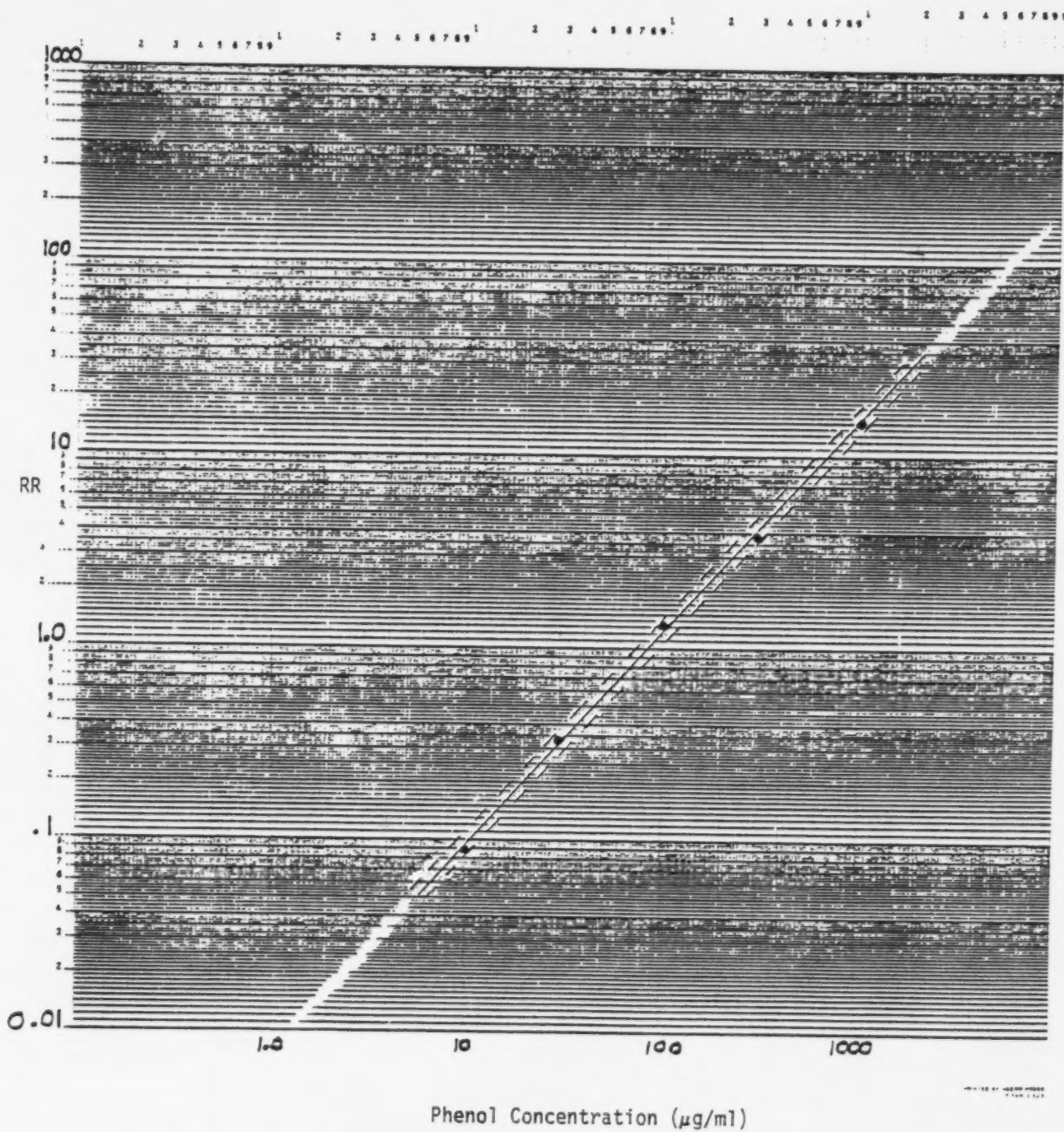


Figure 1

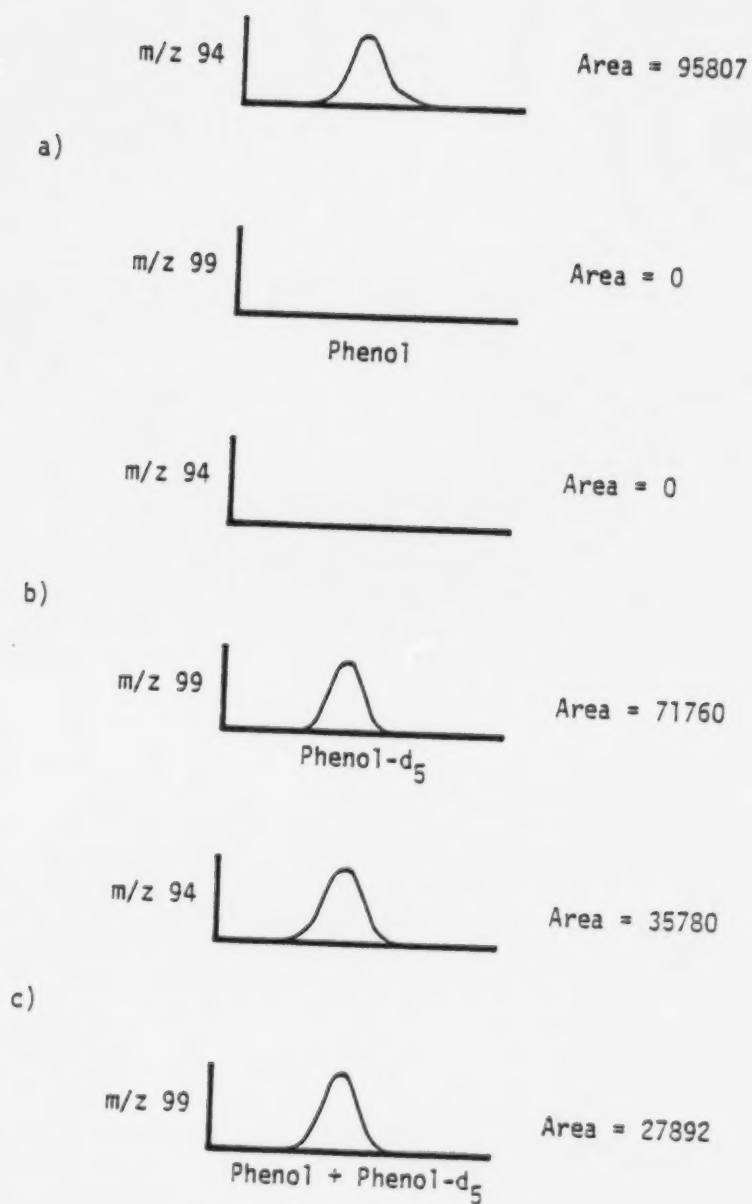


Figure 2. Extracted Ion Current Profiles for a) Phenol, b) Phenol-d₅, and c) a Mixture of Phenol and Phenol-d₅.

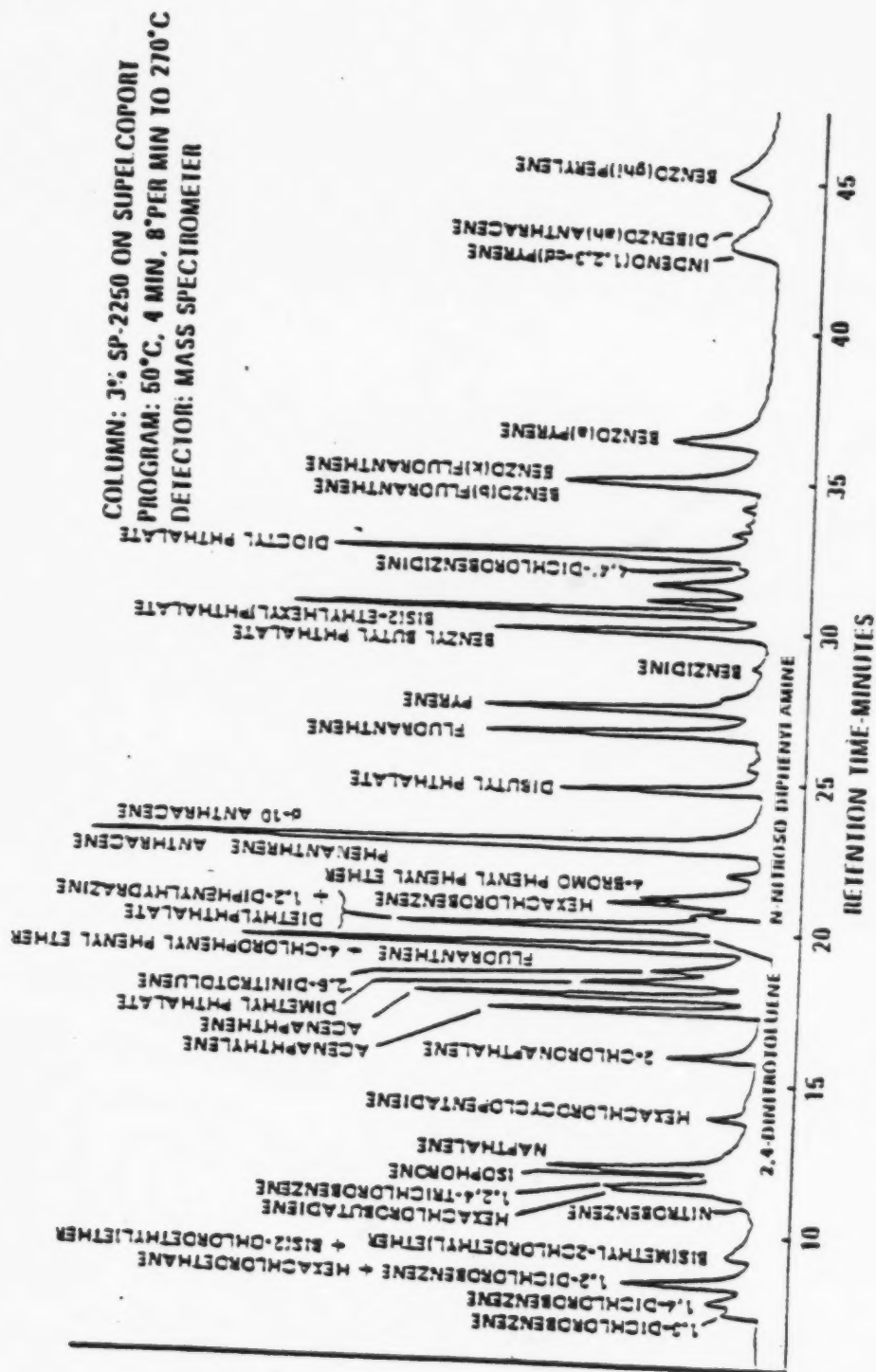


Figure 3 Gas chromatogram of base/neutral fraction

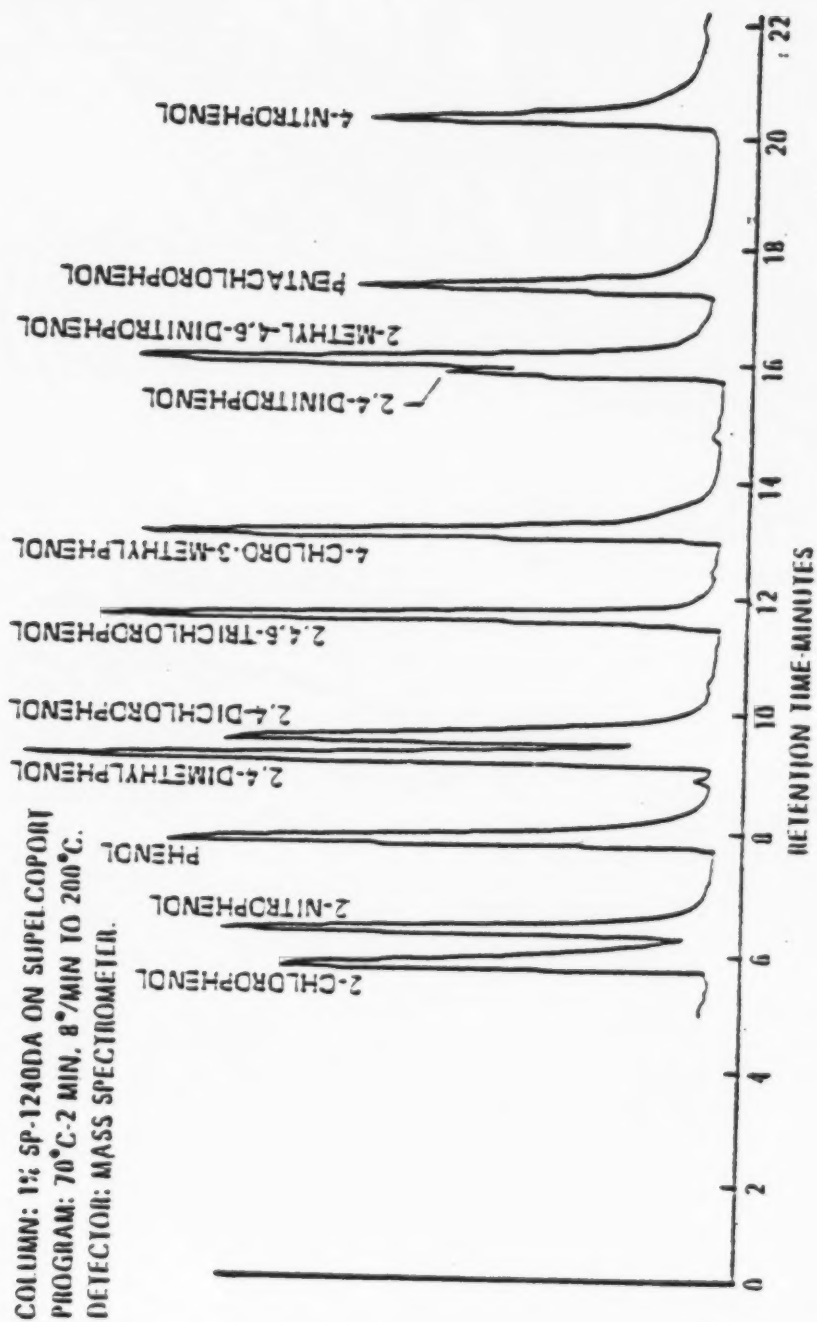


Figure 4 Gas chromatogram of acid fraction